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OCCURRENCE OF PHYSIOLOGIC RACES OF WHEAT RUSTS IN INDIA DURING 1949-50

R.S. VASUDEVA, V.C. LELE AND L.M. JOSHI

(Accepted for publication, June 10, 1952)

Analysis of rust samples obtained from different parts of the country for occurrence of physiologic races is one of the major items of investigation under the Co-ordinated Wheat Rust Control Scheme. Every year samples are supplied by different States to the Rust Research Sub-station, Simla, for analysis of the physiologic races. Black and Brown Rust collections are analysed during summer months and rainy season, and Yellow Rust collections from November to March when conditions are congenial for the study of this rust. It is desirable that the results of analyses are made available regularly to the Mycologists and Plant Breeders in connection with their investigations for breeding for resistance to rusts. The results of analyses of samples collected from 1949-50 crop are recorded here.

Black Rust of Wheat and Barley:—Sixtyfour collections of Puccinia graminis tritici (Pers.) Erikss. and Henn., 62 from wheat and 2 from barley, were analysed using the standard differential hosts and methods (Stakman and Levine, 1922). Only 4 races out of 9 hitherto recorded in India were met with. Eleven collections yielded a mixture of 2 races and 53 a single race. Races 21, 42 and 40 were picked up, 46, 15 and 13 times respectively, whereas race 34 occurred only once. Race 21 which was most widely distributed was recorded from Himachal Pradesh, Delhi, Uttar Pradesh, Bihar, Madhya Pradesh, Madhya Bharat, Bombay and Hyderabad (Deccan), and race 42 from Mysore and all the above mentioned States except Delhi and U.P. It is interesting to note that race 15 which at one time was the most prevalent race has become uncommon in recent years. On the other hand race 21 which up to 1936 was rare has come to occupy the foremost position with regard to prevalence and distribution. Races 24 and 75 have not yet been recorded for many years and obviously have become very rare. Races 'A' and 'B' isolated for the first time in 1946 were not picked up.

Brown Rust of Wheat:—Only 19 collections of Puccinia triticinal Eriks. were analysed using the differential hosts selected by Johnston & Mains (1932) and 7 races out of 8 hitherto recorded in India were met with. Ten collections yielded a single race whereas 9 gave a mixture of 2 races. Races 63, 20, 108 and 11 were picked up 12, 8, 3 and 2 times respectively, and races 10, 26 and 106 only once. The most common race 63 was recorded from Himachal Pradesh, Delhi, Uttar Pradesh, Bihar, Madhya Pradesh and Bombay. Race 107 was not met with.

Yellow Rust of Wheat and Barley:—Twentynine collections of Puccinia glumarum (Schm.) Erikss. & Henn. were analysed on standard differential hosts (Gassner & Straib, 1934) out of which 24 had been collected from wheat and 5 from barley. Eight races out of 10 hitherto recorded in this country were met with. Races 19, 'A' and 31 were picked up 11, 8 and 3 times respectively; races 'G' and 'H' 2 times, and races 13,

TABLE I Distribution of Rust Races in Different States During 1949-50.

	Black Puccinia			Rust :	Yellow Puccinia į	Rust:
States.	No. of samples analysed	Races met with.	No. of samples analysed	Race met with.	No. of samples analysed	Races met with.
WHEAT					The state of	
Punjab (I) Himachal Pradesh.	4	21, 40 & 42		20 & 63	7	A 19, 20, 31, A & H
Delhi. Uttar Pradesh.	1 5	21 21 & 40	1 8	63 & 108 20, 26, 63 & 108	1 11	E 19, 31 & A
Bihar.	7	21, 40 & 42	5	11, 20, 63 &106	2	19 & A.
Madhya Pradesh.	22	21, 40	. 3	10, 11,	2	19
Madhya Bharat. Bombay.	1 10	& 42 21 & 42 21, 34,	-1	$\frac{20 \& 63}{20}$		=
Hyderabad.	11	40 & 42 21, 40	-		1 1	_
Mysore.	1	& 42 42	-	-	-	_
Total	62	21, 34, 40 & 42	19	10, 11, 20, 26, 63, 106 108	24	19, 20, 31, A,* E* & H.**
BARLEY	Carpon Co.				1 22 11	
Punjab Uttar Pradesh Bihar		<u>-</u> 21	Ξ	=	3 2 —	19 & G 13 & 19 —
Total	2	21		_	5	13, 19 & G.**

<sup>Detailed account of these races has been supplied by Mehta (1940).
New Race described on page 129.</sup>

20 and 'E' only once. Race 19 which was widely distributed was recorded from Himachal Pradesh, Uttar Pradesh, Bihar and Madhya Pradesh and race 'A' from Himachal Pradesh, Punjab, Uttar Pradesh and Bihar. The barley collections from Punjab yielded races 19 and 'G' and those from U. P. yielded races 13 and 19. The distribution of the different races is summarized in table I.

Only 112 collections have been studied from the 1949-50 crop. Considering size of the country and distribution of the wheat area, the number of samples analysed is far from adequate and cannot be expected to give a full picture of the distribution of races. It is intended to intensify work in the direction and obtain collections for analyses from different wheat-and barley-growing localities in the country in a more systematic manner. This can only be achieved by careful planning and by obtaining samples from different districts of each State, the number of samples depending on the cultivated area keeping in view the varietal position. If analysis of rust samples is carried out for a few years on these lines we shall be able to obtain reliable picture of the distribution and recurrence of races of wheat rusts. This information is of fundamental importance for a sound programme of breeding for rust resistance.

Division of Mycology and Plant Pathology Indian Agricultural Research Institute New Delhi.

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INFECTION CAUSED BY THE OOSPORES OF SCLEROSPORA GRAMINICOLA (SACC.) SCHROET. ON PENNISETUM TYPHOIDES STAPF AND HUBBARD

D. SURYANARAYANA

(Accepted for publication December 6, 1952)

Introduction

Green ear disease of bajra (Pennisetum typhoides Stapf and Hubbard) caused by Sclerospora graminicola (Sacc.) Schroet. is the most common disease occurring in all the bajra-growing tracts of India and is responsible for considerable losses. Mitter and Tandon (1930) estimated average loss at about 45% though in severely infected fields not a single healthy ear is produced. The total area under bajra is 22.42 million acres and it is the staple food of the masses, so that the crop is of vital importance as far as the food economy of the country is concerned. Even though it has been recognised that the disease is a limiting factor in the cultivation of the crop, systematic study of this disease has not so far been taken up particularly because of the difficulty in germinating the oospores and in determining the period of their dormancy. The earlier work was chiefly confined to the morphological aspect. Butler (1907) made a study of the symptoms and morphology of the fungus while Kulkarni (1913) made further observations on the disease. Chaudhuri (1932) showed that oospores are responsible for infection but the mode of infection as also recurrence of the disease under field conditions which is important from the point of view of evolving control measures of the disease have not so far received adequate attention. The investigation reported in this paper is confined to some of the points relating to these aspects.

Butler (1918) stated that the oospores of this fungus on bajra could not be germinated. Chaudhuri (1932) however, germinated the oospores following the technique described by Hiura (1930) and made a few inoculations on the leaves of growing bajra plants. Butler (1918) reported that mycelium could not be detected in the sound grains collected from partly-formed green ears, nor could the disease be checked when such grains were sown after giving them hot water treatment at 65°C for five minutes. Peglion (1910) in Italy and Weston (1920) in U.S. reported the presence of internal mycelium of Sclerospora in the grains of infected wheat and corn plants respectively. Weston stated that such grains of corn did not give rise to infected plants while Peglion found mycelium in the young parts of the plants raised from grains collected from diseased heads. Melhus and Van Haltern (1925) as also Tasugi (1935) found that oospores of Sclerospora on Setaria could overwinter in soil and cause fresh outbreaks of the disease. The mode of infection caused by oospores of Sclerospora on Setaria was shown to be through the underground parts by McDonough (1938).

MATERIAL AND METHODS

The infected material was collected from fields near Agra and also obtained from Poona and Coimbatore during 1943-48. It was divided into

three parts and was stored until the time of inoculation (a) inside the laboratory; (b) outside the laboratory exposed to atmospheric conditions (c) and in pots buried in fields. The pots before burying were covered with a piece of cardboard and cotton wool. Seeds of bajra of a local variety from Agra were throughout used in these experiments and for inoculation purposes, 12" pots, previously washed and cleaned with water were taken and filled with soil sterilized before hand by steaming in an autoclave. Oospore material was germinated by the method described by Hiura (1930). Seedlings were raised in sterilized soil infected with inoculum. To carry out inoculations through soil, grooves about an inch deep and one and a half inch apart were made and oospore material was uniformly added. About 50 seeds were sown in each pot; more oospore material was added over these seeds and the grooves were finally covered with soil. The second type of inoculation was carried out as described by Chaudhuri (1932). piece of infected leaf bearing oospores was dipped in a solution of mercuric chloride (1 in 500) for five minutes. It was then thoroughly washed in several changes of distilled water and teased in a tube containing sterile distilled water. Loopfuls of spore suspension were placed on the leaves of the experimental plants and in a few cases the leaves were also pricked before the spore suspension was placed on them. The plants were covered to provide high humidity for 24 hours and later removed to the glass house.

EXPERIMENTAL RESULTS

A. Infection tests:

With a view to determine the influence of the conditions of storage of the inoculum a series of soil infection tests were conducted. The symptoms of the disease namely stunting, chlorosis, sporangial and oospore production were observed in some of the inoculated plants (Plate I, II-figs. 1-4). The results relating to the storage conditions are summarised in Table I.

TABLE I

Infection in relation to storage conditions

		Dis	eased m	aterial	stored	in			
La	borato	ry	At	mosphe	ere		Soil		
	Plants	W-W	Plants		Plants				
inocu- lated	infec- ted	%inf- ection	inocu- lated	infec- ted	%inf- ection	inocu- lated	infec- ted	%inf- ection	
36	0	0	52	1	1.9	33	2	6.0	
$\begin{vmatrix} 350 & 6 \\ 250 & 4 \\ 300 & 1 \end{vmatrix}$	6	1.7	200	7	3.5	200	2	1.0	
	4	1.6	250	2	0.8	250	4	1.6	
	1	0.3	100	1	1.0	100	0	0	
350	20	5.7	350	0	0	1100	81	7.4	
i	36 350 250 300	Plants noculated infected 36 0 350 6 250 4 300 1	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Plants	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Plants Plants Plants Plan	Plants Plants Plants Plan	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	

No definite conclusion can be drawn from the infection tests conducted with diseased material stored under different conditions but excepting in the set of experiments conducted in 1947 there is a tendency of slightly higher infection with material stored in soil.

The infectivity of the diseased material collected from centres other than Agra *i.e.* Poona and Coimbatore was compared with the local material collected at Agra. All the samples after collection were stored under different conditions as stated earlier. The results of the experiments are set out in Table II. The material from Coimbatore failed to produce infection.

TABLE II

Infection with inoculum obtained from different stations

Station	Mean % of in	fection under different storage of inoculum	nt conditions
	Laboratory	Atmosphere	Soil
Agra	2.4	1.1	5.3
Poona	2.0	Nil	6.0
Coimbatore	Nil	Nil	Nil

In another set of experiments infection tests were carried out on the lines described by Chaudhuri (1932) by keeping a suspension of oospores on the young leaves of bajra seedlings. Altogether 86 seedlings 6, 8, 10 and 12 days old were inoculated. All such attempts to produce infection were unsuccessful even though the oospore material showed germination from 4-6 percent.

B. Recurrence of the disease:

Apparently sound grains left over in some of the affected ears may harbour the mycelium of the pathogen and form a source of infection. In order to verify this, plants were raised from such grains and at the same time some of them were fixed in Rawlin's (1943) F.A.A. (50% ethyl alcohol 100 e.c., formalin 6.5 e.e., acetic acid 2.5 e.e.) for a week, washed, dehydrated and embedded in paraffin. Sections were cut at 9 μ , stained with iron alum haematoxylin and examined for internal mycelium. Butler's findings regarding the absence of mycelium in such grains was confirmed though septate mycelium in some of the grains was observed but the plants raised from such a lot of seed were normal showing thereby that the seed does not harbour the mycelium of the causal organism and that the disease is not carried through seed.

C. Histological details of injection:

To study the details of infection grains of bajra were rolled in fine oospore material and sown between moist wads of cotton wool placed inside a pair of Petri dishes. To ensure maximum infection some obspore material was also dusted on the seeds. Inoculated seeds were sown in sterilized soil in order to study the details of infection of plants of more advanced age. Infected plants of different ages obtained therefrom were cut into small pieces; fixed and stained for examination. Inter and intracellular mycelium in the adventitious roots, base, middle and apex of the stem were observed. Hyphae were confined to the parenchymatous parts of the stem occurring rarely inside the xylem elements while haustoria were observed frequently. When once inside the stem, hyphae reached the growing point of the stem wherefrom they spread out further into the unfolding leaves and spikelets (Plate II, figs. 1-4). Brown necrotic patches containing hyphae were seen in the tissues of various parts of the infected plants and at times the entire shoot apex became necrotic. In a few cases the pistil was replaced by a small axis bearing numerous protuberances (Plate IV, figs. 1-4).

DISCUSSION

Infection secured in these experiments is low and erratic although Melhus and Van Haltern (1925) and Tasugi and Akaishi (1935) reported high percentages of infection when they inoculated corn and Setaria respectively with oospores of Sclerospora on Setaria. Low infection is obviously due to poor germinating capacity of the material which may be due to variations in weather conditions to which the oospores are subjected and to their maturity. Work in this direction is however, necessary. Oospores in soil caused disease even after long periods of 1-3 years of storage withstanding successfully the high temperatures obtained at Agra. The maximum temperature in shade recorded during this period was 115°F.

ACKNOWLEDGEMENTS

Grateful acknowledgements are due to the late Dr. K.C. Mehta under whose guidance this work was carried out at Agra. I am also grateful to Dr. R. S. Vasudeva, D.Sc., Ph.D. (Lond.), D. I. C., F. N. I., Head of the Division of Mycology, Indian Agricultural Research Institute and Dr. S. Sinha, Professor of Botany, Agra College, for critically going through the manuscript.

SUMMARY

Infection caused by oospores of Sclerospora graminicola on bajra (Pennisetum typhoides) has been shown to be low and erratic.

Oospores stored under different conditions have been found to be infectious for a period of 1-3 years.

Oospores initiate infection in the underground parts of bajra from where it spreads upwards.

Division of Mycology, Indian Agricultural Research Institute, New Delhi—12.

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EXPLANATIONS OF PLATES

PLATE I

An infected plant showing stunted appearance. The upper leaves are chlorotic.

PLATE II

- Fig. 1. Artificially infected plants.
- Fig. 2. Oo spores inside the tissues of the leaf of an affected plant. ≥ 250 .
- Fig. 3. A group of sporangiophores emerging through a stoma. X 420
- Fig. 4. A sporangiophore showing sterigmata and two sporangia attached, X:420.

PLATE III

- Fig. 1. Hyphae at the shoot apex and at the base of an unfolding leaf. $\times 225$
- Fig. 2. Intra cellular hyphae in the cells. X 250
- Fig. 3. Hyphae in the young inflorescence apex. X 150
- Fig. 4. Hyphae in the spikelets of the green ear. X 150

PLATE IV

- Fig. 1. Shoot appex of an infected plant showing necrosis. X 60
- Fig. 2. A longitudinal section of the spikelet of a green ear showing leafy outgrowths surrounding a central axis bearing small protuberances representing the pistil. X 60
- Fig. 3. A portion of the green ear showing brown necrotic cells. X 60
- Fig. 4. A magnified part of the same showing hyphae ramifying inside. X250.



PLATE I



PLATE II

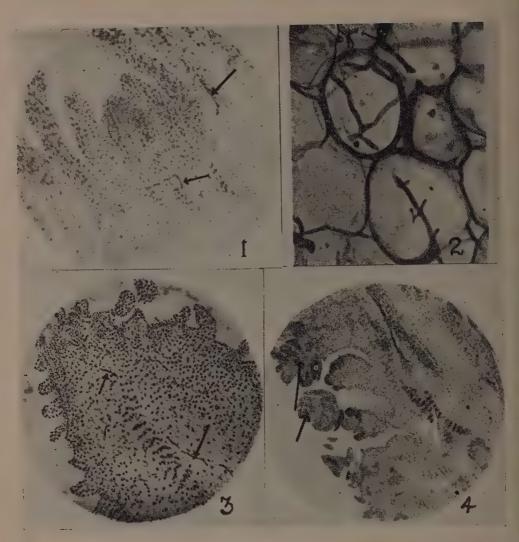


PLATE III



PLATE IV

BACTERIAL LEAF-SPOT OF CASSIA TORA L.

Y. S. KULKARNI, M. K. PATEL & G. W. DHANDE.

(Accepted for publication December 15, 1952)

Tender compound leaves of *Cassia tora*, an annual shrub growing along the roadside and in waste lands throughout India are boiled and eaten and the pods used for medicinal purposes against skin diseases. Tons of seeds are used for the preparation of a blue dye in South India but what has made it popular, is its roasted seeds substituting coffee. A bacterial leaf-spot on this host was noticed at several places in Bombay State in September, 1948 and this paper forms a detailed account of a short note by Kulkarni *et al* (1951).

SYMPTOMS OF THE DISEASE

On leaves: The pathogen produces a few, round, watersoaked spots (1mm.) surrounded by a distinct halo (2mm.) (Plate I, fig 2) which increase to 5mm. in a fortnight and resemble "tikka" of groundnut (*Cercospora personata*). Bacterial shining beads or fine scales are found on the under surface of spots which when coalescent become irregular in shape and rugose. Badly affected leaves become yellow and are easily shed.

On petioles, stems and branches: Under favourable conditions, mid and side veins of leaves get infected. Infection extends to the petioles and down to the tender stems, forming on the latter vertical, gray streaks 5cm. long which gradually become deep brown or black.

On pods: Round to irregular, water-soaked spots are common all over the pod which looks as if it is hard pressed either at the base or at the tip or sometimes at both the ends. The infected portions get constricted and become sooty.-black (Plate I, fig 3 B). The unaffected portion of the pod, however, remains normal and is filled with seeds.

On seeds: Seeds from infected pod are small and shrivelled with a small black spot on the seed coat.

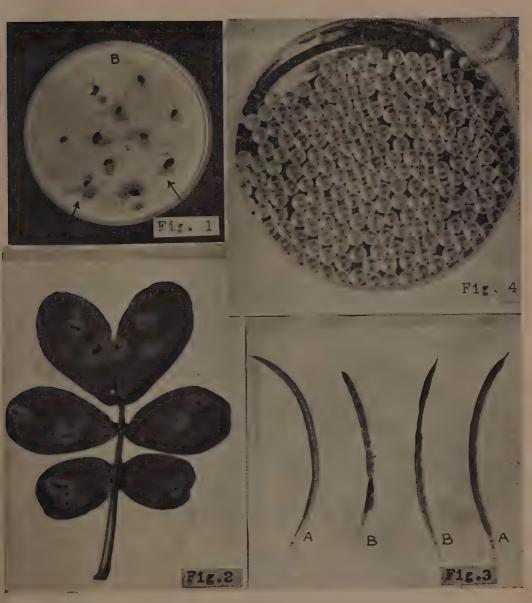
ISOLATION OF THE CAUSAL ORGANISM

The pale yellow organism was easily isolated by ordinary plate method and pathogenicity proved.

Influence of temperature on growth: In order to find the minimum, optimum and maximum temperatures for the growth of the organism, inoculated slants of potato dextrose agar were incubated at 10° , 15° , 20° 26° , 31° , 35° and 40° C. The growth at the end of 6 days shows that the organism has a wide range of temperature, making best growth at temperature range of 26° - 31° C. but failing to grow at 40° C.

Thermal death point: About 51°C.

Effect of exposure to the sun: Agar slants inoculated and exposed to sun for 10 minutes remained sterile.



Explanation of Plate I:—Fig. 1. Infected seeds of C. tora yielding a pure culture of X. cassiae on agar plate. Fig. 2. Leaf of C. tora (natural size) showing chlorotic area round bacterial leaf-spots. Fig. 3 A. Healthy pods of C. tora. B. Constriction of pods due to bacterial infection (1/2 natural size). Fig 4. Glass beads used for the desiccation experiments, (Full size).

Morphology and Staining Reactions

The organism is a short rod usually single, Gram-negative, not acid fast and a non-spore former. In 48 hour old culture on potato dextrose agar, it measures 1.2 to 2.1x0.8 to 1.0 μ . It stains readily with Ziehl's carbol fus chin.

Motility: Motile in a hanging drop of peptone broth. Stained by the method developed by Patel, Kulkarni and Gaikwad (1950), it was found to possess a polar flagellum.

Capsule stain: The Hisse's method for staining showed it capsulated.

CULTURAL AND PHYSIOLOGICAL CHARACTERS

The cultural characters of the organism were studied on various media prepared following the procedures laid down in the Manual of Methods using ingredients of the highest purity. They were incubated at $27^{\circ}-30^{\circ}\mathrm{C}$.

Potato dextrose agar slants : Growth copious, raised, smooth, shining, filiform and butyrous.

Potato dextrose agar plates: Colonies smooth, circular with lobate margin, shining, convex, butyrous, pinard yellow (R) with a diameter of 1.2 cm. after 7 days with striations only at the periphery.

Potato cylinders: Growth copious and covering the entire surface in 4 days, maize yellow (R) while the cylinders changing to ecru drab (R) in 8 days.

Nutrient agar slant: Growth poor, flat and filiform.

Nutrient agar plates: Colonies flat, glistering with fringed margins, wax yellow, diam. 5 mm. after 4 days.

Nutrient dextrose agar plates: Colonies round, convex with entire margins, primuline yellow, diam. 1.2. cm. in 4 days.

Nutrient dextrose broth: Good growth with a pellicle, colour unchanged and odour absent.

Yeast-Glucose-Chalk Agar: Growth copious, shining, butyrous and maize yellow. Quite suitable for stock cultures as the organism remains viable for 3 months.

Gelatin: Liquefied.

Starch: Hydrolysis is evident after 4 days.

Growth on casein, egg albumen and cellulose agars: Good growth on casein and egg albumen but unable to utilise cellulose.

Lipolytic medium: Agar plates prepared according to Starr and Burkholder (1942), inoculated and incubated for 6 days showed absence of deep blue colour (pH acid) round the colonies indicating absence of lipase, a finding somewhat contrary to that of Dowson (1949) who states that *Xanthomonas* spp. possess marked lipolytic activity.

Litmus milk: Moderate reduction of litmus and partial digestion of casein.

Hydrogen sulphide: Produced.

Ammonia: Positive.

Indole: Not produced.

Nitrates: Not reduced to nitrites.

Voges-Proskauer and Methyl-Red Tests: Negative.

Loeffler's Solidified Blood Serum: Liquefaction after 10 days.

Citrate: Not utilised as a source of carbon.

Patel's (1926) medium: After 4 days, the colonies circular with lobate margins, shining with a halo.

Koser's uric acid medium: No appreciable growth.

Tolerance to sodium chloride: Up to 3 per cent.

Endo's agar: Good growth without any metallic sheen or red colour around the colony.

Synthetic Asparagin Medium: No appreciable growth.

Utilisation of Carbon Compounds

Lewis (1930) working on the nutritional requirements of 14 strains of X. malvacearum found that 25 carbon compounds failed to support growth of the organism in the absence of nitrogen. The accurate fermentation studies of carbohydrates are of great help in identifying and classifying bacterial organisms when their morphological and physiological characters are very nearly identical. Thus Burkholder (1932) could distinguish closely related species of the genus Phytomonas on the basis of their ability to ferment several carbohydrates and emphasised that beef peptone broth was unsuitable as a basal medium.

The organism could utilise dextrose, sucrose, lactose, maltose, dextrin, glycerol, mannitol while it made slight to no growth in rhamnose, arabinose, raffinose, salicin, dulcitol, ethyl alchol and acetic, oxalic and tartaric acids. It, however, grew well on the medium containing citric acid.

Utilisation of Nitrogen Compounds

Ostroff and Henry as quoted by ZoBell (1946) have shown that whereas all the 15 representative aerobic bacteria of marine origin were able to utilise peptone, only 8 could utilise asparagin and only 5 could utilise di-ammonium phosphate as a source of nitrogen. The work on the nutritional requirements of micro-organisms has mostly been confined to saprophytic fungi with scanty attention paid to pathogenic forms and still less to the phytopathogenic bacteria. Burkholder (1939) has shown that *Phytomonas* spp. could grow well in media containing monobasic ammonium phosphate as the only source of

nitrogen. Patel and Kulkarni (1949) showed that ammonium citrate, ammonium nitrate, ammonium sulphate and potassium nitrate when added singly to the medium containing sucrose supported growth of *Xanthomonas malvacearum*. The organism under study grew well in a synthetic medium containing separately potassium nitrate, monobasic ammonium phosphate, ammonium citrate and ammonium nitrate.

Bhide (1948) got striking differences in the utilisation of amino acids by two species of *Xanthomonas*, *Phytobacterium* (*Pseudomonas*) solanacearum, *X. stewartii* and four species of *Aplanobacter* (*Corynebacterium*), the last named being very inactive in the utilisation of amino-nitrogen. He (1949) reported that the virulent strain of *A.* (*C.*) michiganense utilised a very large number of organic compounds especially amino acids as sources of nitrogen when carbon was supplied in the form of dextrose while the weakly virulent strain could utilise only aspartic and glutamic acids.

In order to study the growth of the organism by adding various amino-acids alone as a source of nitrogen and carbon in one series and in another with one per cent dextrose as a source of carbon, the inorganic basal medium as advocated by Mushin (1938) was prepared. The pH was adjusted after adding the amino acids at the rate of 0.1 per cent except tryptophane which was used at the rate of 0.01 per cent. The following amino acids were used (table 1). Cystine was dissolved in dilute hydrochloric acid before adding to the basal medium. The observations recorded after a week are given in table 1.

Table 1
Utilisation of Organic Nitrogen with and without Dextrose

Source of nitrogen	With dextrose	Without dextrose
Glycine Tyrosine Tryptophane Cystine Arginine Aspartic acid Glutamic Acid Creatine Histidine Guanidine hydrochloride Proteose peptone (check)	++ + + + + ++ ++ ++ ++ ++ ++ ++	 + +

Note: +++ Excellent growth, ++ Good growth, + Medium growth and -- No growth.

It will be noticed that most of the amino acids do not allow growth of the organism in the absence of dextrose indicating that it can obtain nitrogen from them but not carbon.

OLYGODYNAMIC ACTION OF HEAVY METALS

Porter (1947) noted aluminium, chromium, nickel and thorium poisonous beyond 100 p.p.m. Patel and Kulkarni (1949) reported that nitrates of copper, iron, lead, nickel, silver and several other metals are lethal not only to *X. malvacearum* but also to saprophytic and parasitic fungi.

In the present study, the following metals were dry-sterilised in Petri plates and mounted, one in a plate. A broth culture of the organism was then poured over the metal. After 2-3 days, the bactericidal action of different metals was visible in the form of round clear zones immediately surrounding the metals. The following table gives the area of zones with reference to different metals used.

Table 2

Olygodynamic action of metals

Metal	Radius of zone in mm.
Silver Copper Nickel Zinc Magnesium Iron Lead Aluminium Manganese	 12 15 5 Slight Slight Slight Nil Nil Nil

INFECTION EXPERIMENTS

One month old *C. tora* plants raised in sterilised soil in earthen pots and kept under a bell-jar for 24 hours in a basin of water when removed were found covered with very fine drops of water. After making multiple punctures and vertical scratches on leaves and stems respectively, the plants were inoculated and again covered with bell-jars for 24 hours. The control plants were sprayed with sterile water only. Both series were then removed to the glass-house benches for further observation.

The first signs of infection appeared after 4 days as small, round, water-soaked spots which rapidly increased in size within a fortnight (Plate I, fig. 2). The organism reisolated from the spots resembled the original culture in all respects. The control plants remained healthy.

Burkholder (1937) working with leaf-spot of Geranium concluded that the best infections occurred with greater certainty and with somewhat shorter incubation period when the leaves were injured previous to inoculation. Burkholder and Guterman (1935) failed to get infection on carnations by Phytobacterium (Pseudomonas) woodsii in the absence of

wounds. To determine whether injury is essential in the present case, the following experiment was performed:—

Cassia tora plants grown in 3 pots for about a month were selected for the purpose. Leaves of plants in one pot were slightly punctured and sprayed with bacterial suspension; the leaves of plants in the second pot were only rubbed on both sides with a cotton swab dipped in the bacterial suspension while the leaves of plants in the third pot were only atomised with the bacterial suspension. All the 3 pots were then kept separately below the bell-jars in water for 24 hours and then removed to the glasshouse bench. Plants with punctured leaves showed water-soaked spots around punctures after 6 days while on the same day the plants with leaves rubbed showed 1 or 2 stray water-soaked spots only. The plants sprayed with the bacterial suspension only, although not showing signs of infection up to 6 days, showed numerous, minute, water-soaked spots all over the leaf surface after 7 days. This shows that although injury to the leaves is sure and quicker method of infection, stomatal invasion invariably occurs on uninjured leaves.

ANTAGONISM

Waksman et al, as reported by Burkholder (1948), concluded that phytopathogenic bacteria do not behave as a group but vary in their reactions to six different antibiotics tried. Brown and Boyle (1945) state that young crown galls were killed when punctured and crude penicillin applied. Rudolph (1946) tested the antibiotic properties of penicillin against Erwinia amylovora and X. juglandis and found that the substance in vitro was not only bacteriostatic but bactericidal as well. Attempts to control both organisms in vivo met with failure. Antibiotics have been proved to be responsible for controlling soil borne pathogens. Thus, Lee (1920) working with X. citri found that it disappears from unsterilised soil usually within 6 days due to antagonistic effect of soil inhabitants. Ark and Hunt (1941) showed that Bacillus vulgatus and an unidentified yellow spore forming bacillus were antagonistic to X. campestris, X. malvacearum etc.

. In order to prove the antagonistic effect of soil and water organisms on the Cassia organism, the following 3 experiments were performed:

- (1) The organism grown in peptone broth in tubes for 8 days was mixed with unsterilised soil which was removed daily by a scalpel, and its suspension sprayed on leaves of *C. tora* in which infection could be obtained only upto 6 days after inoculation of the soil showing the antagonistic effect of soil micro-organisms. It however remains viable in sterilised soil for 210 days.
- (2) Unsterilised well water in one case was inoculated with the organism while in the other, it was sterilised previous to inoculation. The water suspension was atomised on the host daily in the case of unsterilised water and at an inteval of 4 days in the case of sterilised. The pathogen could remain infective for 30 days in sterilised water as against 3 days in the unsterilised water showing the antagonism of bacteria in unsterilised well water.

(3) In this experiment, the Cassia organism and Phytobacterium (Pseudomonas) mangiferae-indicae were grown together in peptone broth to find substances antagonistic to each other. Typical leaf-spots were produced on both the hosts after 8 days showing absence of antagonism between them.

RESISTANCE TO DESICCATION

Smith (1901, 1905-14), Ralph (1920) and Edgerton and Moreland (1913) found that *X. phaseoli* remains alive for 70, 18 and 200 days respectively, when dried on glass covers at room temperature. Uppal, Patel and Nikam (1946) and Patel and Diwan (1950) showed that *X. phaseoli* var. *indicus* and *X. vignicola* growing in peptone dextrose broth, when transferred on sterile cover slips, remained alive for 17 and 7 days respectively. *X. malvacearum* according to Patel and Kulkarni (1950) was found to resist desiccation for 16 days at 31°C. Cabral (1944) working with *X. begoniae* used glassrods instead of cover slips and showed that it could survive 75 days at 25°C.

A week old peptone broth culture of the organism was poured aseptically over 100 sterilised round glass beads in a Petri dish (Plate I, fig. 4); the broth was drained and the beads transferred to another sterile Petri dish. At regular interval of 2 days, 2 beads from the dish were dropped aseptically in a tube of peptone broth, observations made for growth and Cassia tora inoculated. It resisted desiccation for 12 days.

TRANSMISSION OF THE DISEASE

Anderson (1926) is lating X. pruni from dead leaves felt that bacteria in gelatinous masses are protected from desiccation. Burkholder (1937) could isolate virulent cultures of bacterial leaf-spot of Geranium from leaves overwintered under mulch and snow for one season. Patel and Kulkarni (1950) showed that X. malvacearum could remain viable in infected cotton leaves at room temperature for 290 days. In order to get reliable data on the possibilities of the transmission of the disease from one season to another, the following experiments were tried:—

Seeds from infected pods of Cassia distinguishable from healthy ones by their light weight and by a black speck on the seed-coat were collected in March, 1950. Four months later, the seeds were disinfected and aseptically dropped in peptone broth. The broth showing cloudy growth after 8 days was atomised on C. tora plants on which typical leaf-spots were produced proving that the infected seeds could be a source of primary infection after 4 months. This experiment was repeated with the difference, however, that the seeds were planted on potato dextrose agar. Out of 76 seeds, 23 yielded typical yellow culture around them (Plate I, fig. 1) which proved pathogenic on inoculation.

For another experiment, infected leaves and stems showing cankers were collected and stored in a tissue paper bag at room temperature for one year. These were then cut and macerated. The material was then thoroughly soaked in water for half an hour and the suspension sprayed on young healthy host plants on which infection was noticed after 8 days.

To the sterilised soil in tubes was added broth inoculated with X. cassiae and kept at 27°-30°C. Every fortnight, a bit of this soil was dropped in nutrient dextrose broth which when atomised every 4 days on young host plants showed infection up to 210 days. It is of interest to note that organisms such as Agrobact. tumefaciens, Chlorobacter-marginatum, X. phaseoli, Pectobact. carotovorum and P. atrosepticum live for more than 500 days in sterilised soil (Patel, 1929).

HOST RANGE

Host range is a matter of considerable practical importance, since some bacterial pathogens infect only one plant while others infect numerous unrelated hosts. In the present studies, an extensive host range including species of *Cassia* and several related genera of Caesalpinae besides plants of several botanically distinct families were included. The seeds were sown separately in sterilised soil in 6" earthen pots and the plants sprayed with the organism when a month old, *C. tora* of the same age serving as a control. The method of infection was exactly the same as described earlier. Observations were made every fourth day for over a month when the plants were finally discarded. A list of plants tried is given below:—

Cassia tora L., C. absus L., C. auriculata L., C. alata L., C. corymbosa Lam., C. didymobotrya, C. fistula L., C. fructiocosa Milt., C. glauca Lam., C. grandis L., C. hirsutum, C. javanica L., C. marginata Roxb., C. mimisoides L., C. moschanta H. B. K., C. nodosa Ham., C. occidentalis L., C. pumila, C. siamea Lam., C. spectabillis DC., Acacia arabica Willd., A. catechu Willd., A. concinna DC., A. leucophloea Willd., Alysicarpus rugosus, Arachis hypogaea L., Caesalpinia sepiaria Roxb., C. pulcherrima Swartz., Cajanus cajan Millsp., Cicer arietinum L., Crotolaria juncea L., Cyamopsis psoraloides DC., Desmodium diffusum DC., D. gangeticum DC., Dolichos biflorus L., D. lablab L., Glycine max Merr., Lathyrus sativus L., Medicago sativa L., Moringa pterygosperma Gaertn., Phaseolus aconitifolius Jacq., P. mungo L., P. mungo var. radiatus L., P. vulgaris L., Pisum sativum L., Poinciana regia Bojer., Sesbania aegyptiaca Poir., S. aculeata Poir., Stizolobium deeringianum Bort., Tamarindus indica L., Trigonella foenumgraecum L., Vigna catjang Walp., Andropogon sorghum Brot., Avena sativa L., Brassica oleracea var. capitata, Capsicum annuum L., Citrus aurantifolia Swingle, Gossypium arboreum L., G. herbaceum L., G. hirsutum L., Hordeum vulgare L., Ipomoea batata Lam., I. muricata R. & Sch., Lycopersicum esculentum Mill., Oryza sativa L., Pennisetum typhoideum Rich., Ricinus communis L., Solanum melongena L., S. tuberosum L., Triticum vulgare Vill., Xanthium strumarium L., Zea mays L.

Of the 20 species of *Cassia* tested, the organism is able to infect only *C. tora*, the original host besides *Pisum sativum*. It fails to infect any of the 31 species comprising 21 genera of the family 'Leguminosae' and 20 others from 8 unrelated families.

Elrod and Braun (1947) believe that all the members of *Xanthomonas* do not represent true species. Wernham (1948) on the other hand has shown pathogenicity to be a remarkably specific character of 17 members of the genus *Xanthomonas* used in cross-inoculation tests on 16 taxonomically distinct hosts. In the present study, 71 species of plants cannot be

considered a narrow range especially since many of them grow side by side. Then, how is it that in repeated trials, the organism infects one or two hosts and not more though of the same genus? The answer may be partly found in the explanation offered by Starr (1946) that although minimal nutritive requirements of any pathogen can be met by the tissues of practically every plant, the pathogen is unable to infect plants other than its host since there exist specific antibiotics capable of inactivating other plant pathogens.

PASSAGE THROUGH A COMMON HOST

Burkholder (1948) argues that the phytopathogenic bacteria have evolved from various soil types or saprophytes on the external surface of plants. This relation to the soil types is shown in the striking resemblance morphologically and physiologically of the Chlorobacter (Pseudomonas) species to the green fluorescent bacteria of the soil. The yellow saprophytes commonly found on plants are also similar in colour to the Xanthomonas species. It is reasonable to believe that certain soil bacteria associating with plants over an infinite period of time may have developed a parasitic nature. It is also possible that non-pathogenic bacteria exist within plants in limited numbers but not produce disease symptoms. Elrod and Braun (1947) are also of opinion that members of the genus Xanthomonas have evolved from a saprophyte either in the soil or on the external surface of plants. This assumption is reasonable in the light of the uniformity of physiological characters of the genus as shown by Dowson (1949). Patel, Dhande and Kulkarni (1951) have shown that these are very much alike in their cultural and biochemical reactions and can only be differentiated on the basis of pathogenicity. Although most members of this genus are host specific, it was found during extensive host range studies that X. cassiae and X. alfalfae could infect pea leaves and pods. The two organisms have also shown closer relationship in serological studies made by Patel, Dhande and Kulkarni (1951).

In order to see whether the two organisms when passed through the common host viz. pea will lose their host specificity, the following experiment was designed:—

Pea plants grown separately in sterilised soil in 2 six inch earthen pots were infected with X. cassiae and X. alfalfae separately with due care. When spots were produced on the leaves of pea by each, reisolation was made from each set and were cross-inoculated on C. tora and Medicago sativa when it was found that the organism isolated from pea infected by X. alfalfae was restricted to Medicago sativa only while organism isolated from pea infected by X. cassiae was pathogenic to C. tora. This shows clearly that even though some of the bacterial plant-pathogens of the same genus have a common host, they are restricted to their own host range even when passed through a common host.

TECHNICAL DESCRIPTION

Xanthomonas cassiae sp. nov.

Organism short rod; average dimension 1.7 x 0.9 μ ; motile by a single polar flagellum; gram-negative; capsulated; optimum temperature for growth between 26-31°C.; thermal death point about 51°C.

On potato dextrose agar plates, colonies smooth, shining, butyrous, colour pinard yellow (R), diameter 1.2 cm. after 7 days; on nutrient agar plates, colonies flat, glistening, colour wax yellow (R), diameter 5 mm. after 4 days; good growth in nutrient dextrose broth within 24 hours; on potato cylinders, copious growth, covering the entire surface; gelatin and Loeffler's blood serum liquefied; litmus milk reduced; casein and starch hydrolysed; ammonia and hydrogen sulphide produced; indol not produced; nitrates not reduced; non-lipolytic; produces acid but no gas from dextrose, sucrose, lactose, maltose and dextrin but not from salicin; M.R. and V.P. tests negative; most of the amino acids not utilised as a source of carbon; sodium chloride tolerant up to 3 per cent; pathogenic to Cassia tora and Pisum sativum.

SUMMARY

A new bacterial leaf-spot on *Cassia tora* was observed at Poona and other places and the causal organism isolated. Water-soaked spots surrounded by a distinct halo first visible on leaves increase in size and become jet black. Bacterial gummy beads are found on the undersurface of spots. Heavily affected leaves become yellow prior to shedding. The petioles and tender stems are infected when the latter crack vertically. As a result of heavy infection, the pod gets constricted at the base or at the tip.

Silver, copper, nickel and zinc produced bactericidal action. Antagonistic substances were not produced.

The organism remains viable in sterilised soil and sterilised well water for 210 and 30 days respectively while in unsterilised soil and unsterilised well water, it is killed after 6 and 3 days respectively. Besides being carried on the seed, it persists in diseased plant trash for one year. It infects Pisum sativum also. It is therefore considered new and the name Xanthomonas cassiae sp. nov. is proposed.

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EFFECT OF STEM RUST, PARTICULARLY ON GRAIN WEIGHT, OF SOME IMPROVED INDIAN WHEATS

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INTRODUCTION

Stem rust (*Puccinia graminis tritici* (Pers.) Eriks. & Henn.) is more destructive than either the brown rust or the yellow rust in the Peninsular India. It causes enormous loss to wheat crop in the quantity and quality of yield when it appears in an epidemic form. Cultivation of rust resistant varieties is the only economical method of control against this disease. Work is already in progress in this direction in the different states and new stem rust-tolerant wheat varieties are now available for cultivation in parts of Bombay and Madhya Pradesh.

During the course of tests of different wheat varieties under artificial epidemic of stem rust in the field at Mahableshwar, marked differences were observed in the severity of infection on Indian improved wheat varieties which were susceptible in the seedling as well as in the adult stages. Hence small scale trials were carried out at Mahableshwar in 1950-51 and 1951-52 to ascertain the relative susceptibility to stem rust of such Indian improved wheat varieties as had previously given indications of tolerance in general tests.

METHOD OF STUDY

The wheat varieties under trial were sown in two series, one under heavy infection of stem rust and the other under sulphur dusting to serve as check. These two series, which will be referred to as 'rusted' and 'dusted' respectively, were about 150 ft. apart since, in similar trials carried out in 1948-49, when these series were side by side, the infection of stem rust was not effectively controlled in the dusted one. The wheat varieties were replicated five times in each series in 'randomised block' design.

It was necessary to decide upon the criterion of judging the relative susceptibility of the different wheat varieties since the damage done by stem rust is due to reduction in the size of the individual grains and in some cases also due to reduction in the number of grains per earhead. The combined effect of both these factors is measured by the total yield. Due to limited field area available for these trials, the plot size was very small (12 to 18 sq. ft. only) and hence the yield per plot could not be relied upon for proper comparison. In their study of the effect of stem rust on yield, quality, chemical composition etc. of Marquis wheat, Greany et al (1941) are of opinion that, of the agronomic characters studied, kernel weight gives the truest measure of damage caused by stem rust. They have also shown that uniform increases in rust severity result in uniform

decreases in the weight of 1,000 grains of that variety. It was, therefore, decided to adopt kernel weight as the basis for comparison and to compare the weight of 1,000 grains of a wheat variety produced under stem rust infection with that of an equal number of grains obtained from rust-free plants in the same field.

Greany (1933) is also of opinion that "in view of the difference in varietal response to disease infection, physiologic forms and to environmental conditions, it is difficult to secure an exact measure of the loss in yield and grain quality from stem rust." This fact has been borne in mind in drawing conclusions from our experiment.

The following ten physiologic races of stem rust were introduced in the artificial epidemic under which the trials were carried out: 15, 21, 24, 34, 40, 42, 42B, 53, 75 and new race 'A.' The varieties in the trials described herein are highly susceptible to all or many of these races in the seedling stage. They were sown about 3 weeks after the major portion of the field area was sown with hybrid progeny under test, with the object of exposing the plants in the trials to infection in the early stage.

Readings on percent severity of infection were made with the help of Cobb's modified scale (Arthur, 1929).

TRIALS IN 1950-51

The following nine varieties were selected for trial: Niphad 4, Mondhya 3-2, A.115, N.P.114-3-2, N.P.710, N.P.715, Motia and A.013. The last two high yielding improved varieties of Bombay and Madhya Pradesh respectively have been observed to be highly susceptible to stem rust every year and hence were included to serve as checks. Other varieties were observed to be less damaged in the general tests of the previous seasons. They were sown in both the series on 13.xi.50. In the rusted series, each plot consisted of 6 rows, each 6 ft. in length while in the dusted one it consisted of 4 rows of the same length. The rows were 6" apart and the plants within a row were 2-3" apart.

Infection of stem rust was first observed in the rusted series on Motia and A.013 on 28.xii.50, *i.e.* about 6 weeks after sowing. A water suspension of urediospores was sprayed on the plants on 6.i.51 and again on 29.i.51, after which the plants were kept covered with thick cloth for about 18 hours. Heavy dew was observed on 7.xii.50 and also for 3 days during the last week of December, 1950. Observasion on the intensity of rust infection were made at intervals of about 10 days until harvest time and a summary of these observations is presented in Table 1.

Infection developed rapidly on Motia and A. 013 and the plants started withering as a result of heavy infection within about six weeks after the initial infection was observed. Compared to these varieties, it developed slowly on M. 3-2 and A. 115 although ultimately they were almost as highly susceptible as A. 013 and Motia. Niphad 4, although more or less free from infection until about the heading time, developed considerable infection afterwards, although it is less susceptible than the four varieties mentioned above. It will be seen from the Table that the average infection on the N. P. wheats is less than on others even at the time of harvest.

Mean per cent severity of stem rust on nine wheat varieties sown in five replications in 1950-51. TABLE 1

	16.iii.51				1		1	1	1	35	31	36.5
	6.iii.51	68.5	Harvested)		001		1	82	44.5	30.7	30	36.2
	22.ii.51	62			69.2	100	(Dried up)	99 °	42	27.	30	27
vation	14.ii.51	52	Dried up		09	86	Many plants)	50 20	. 38.4	17.4	17	21
-Date of observation-	7.ii.51	45	100	(Withering)	. 94	68.4		43	27.2	15	11.4	14.4
	25.i.51	22.3	71		32	45	-	О	17	7 (D)	D,	11
	15.i.51	T	26.4	leaf blades)	1.8	11.2		c 1	H	Ħ	T	H
,	6.i.51	H	22		Ħ	H		D	H	0	0	0
	28.xii.50	0	H	(0	H		0	0	0,	0	0
	Variety	Niphad 4 *(11.i.51)	Motia (11.i.51)	(TO::::2)	Mondhya 3-2 (20.i.51)	A.013 (20,i.51)		A.115 (15.i.51	N.P.52 (18.i.51)	N.P.114-3-2 (20.1.51)	N.P.710 (15.i.51)	N.P.715 (15.i.51)

*The date in brackets denotes the time of heading; T — Trace; D—The infection was developing when the observation was made.

In the 'dusted' series, the plants were dusted with fine sulphur at about 30 lbs. per acre twice a week soon after the emergence of seedlings. Stem rust was effectively controlled except on A. 013 and Motia, of which the border plants showed 5-10 per cent infection late in the season. Other varieties showed infection in traces only. The weight of 1,000 healthy grains was calculated for each replication from the number of grains contained in 50 gms. taken at random from the produce of rust-free plants. The mean of five replications was used for calculating the loss in grain weight in the rusted series.

Weight of 1,000 grains was calculated for each replication in the 'rusted' series in the same manner as that for the dusted one. Where the total yield of a replication was less than 50 gms. the weight of 1,000 grains was calculated from that of the actual number of grains.

The weight of 1,000 grains for each replication in the rusted series and the loss in grain weight, expressed as a percentage of the mean weight of an equal number of healthy grains are shown in Table 2.

Table 2

Weight of 1,000 grains, in gms., of nine wheat varieties obtained in 1950-51 under infection of stem rust and the loss in grain weight, expressed as a percentage of the weight of an equal number of healthy grains.

Variety	†R. I	R. II	R. III	R. IV	R. V	Mean
Niphad 4	a. 12.87	12.11	10.70	12.01	13.77	12.29
*(45.14)	b. 71.48	73.17	76.29	73.39	69.49	72.76
Motia	a. 4.46	2.88	2.06	3.07	4.72	3.44
(49.90)	b. 91.06	94.23	95.86	93.85	90.54	93.11
Mondhya 3-2	a. 8.36	5.46	5.18	7.79	6.10	5.98
(43.34)	b. 80.71	87.40	88.05	88.95	85.93	86.21
A.013	a. 2.57	2.08	1.74	2.10	2.44	2.18
(45.83)	b. 94.39	95.46	96.21	95.42	94.68	95.23
A. 115	a. 6.44	6.38	4.66	4.94	4.08	5.30
(42.17)	b. 84.73	84.87	88.95	88.28	90.32	87.41
N. P. 52	a. 19.77	19.37	18.20	22.13	18.73	19.64
(36.89)	b. 46.41	47.49	50.69	40.01	49.20	46.76
N. P. 114-3-2	a. 29.81	30.65	28.55	31.01	30.07	30.02
(42.69)	b. 30.17	28.18	33.12	27.35	29.56	29.67
N. P. 710	a. 33.52	33.22	35.59	34.99	34.09	34.28
(43.02)	b. 22.08	22.78	17.27	18.66	20.76	20.31
N. P. 715	a. 30.23	33.09	34.64	33.01	31.86	32.57
(43.32)	b. 30.22	23.61	20.04	23.77	26.46	24.82

[†] R stands for replication.

^{*} The figure in brackets denotes the mean weight of 1000 grains in gms., obtained from the dusted series (mean of five replications).

a. Weight of 1000 grains in gms., obtained under infection of stem rust.

b. Loss in grain weight expressed as a percentage of the weight of an equal number of healthy grains.

Analysis of variance (Per cent loss in grain weight)

Factor	D. F.	Sum of squares	Variance	F.
Total	44	39371.38		
Varieties	8	39089.82	4886.22	1130.02 **
Blocks	4	143.20	35.80	
Error	32	138.36	4.324]

The critical difference between any two variety means is 3.769 per cent. (P=.01).

Conclusions

A.013, Motia, A.115, M.3-2, N.4, N.P. 52, N.P. 114-3-2, N.P. 715, N.P. 710

The varieties have been arranged in the decending order of the mean per cent loss in grain weight shown in Table 2. The order of superiority of the varieties is therefore in reverse direction. Thus N. P. 710 is superior to others.

TRIALS IN 1951-52

The object of these trials was to repeat those of the previous year under more rigorous conditions of rust infection, particularly during the seedling stage. Mondhya 3-2 was dropped from these trials since it proved almost as bad as Motia and A.013 in the previous year. Although A.115 was highly susceptible in that year, it was often observed to be less susceptible than A.013 in previous years and was therefore retained. There were thus eight varieties, viz., Niphad 4, Motia, A.013, A.115, N.P. 52, N.P.114-3-2, N.P.710 and N.P.715. These were sown in two series on 9.xi.51, the number of replications, plot size, the distance between rows, etc., in each series being the same as in 1950-51.

In the rusted series, the seedlings were sprayed with a suspension of unrediospores of a mixture of ten physiologic races of stem rust on 20.xi.51, i. e. about 6 days after germination and this was followed by cloudy and drizzly weather for about 3 days. Infection of stem rust was noticed in traces on Motia and A.013 on 28.xi.51 and 1.xii.51 respectively. The initial infection thus started within about 3 weeks from sowing. It will be recalled that in the trials carried out in 1950-51, the initial infection was observed 5 to 6 weeks after sowing. infection developed rapidly on A.013 and Motia as in 1950-51 and the leaf blades and leaf sheaths were heavily infected when these varieties were in heading or in flowering stages. On the other hand, the infection on the leaf blades of N.P. wheats, Niphad 4 and A.115 was either in traces or very light upto that period. During the post-heading period, however, Niphad 4 and A.115 were much more susceptible than the N.P. wheats which seem to possess distinct tolerance to stem rust as compared to others. The mean percent severity of infection on nine different dates of observation is shown in Table 3.

In the dusted series, the plants were treated with sulphur twice a week as in 1950-51. All varieties were more or less free from rust infection for over three months. As a result of very heavy dew which occurred on

TABLE 3.

Mean per cent severity of stem rust infection on different dates on eight wheat varieties, sown in five replications in 1951-52.

28.xi.51 3.xii.51 17. 0 T on 10- 15% plants T 2.5 on 8%	11	Mean 2 2 2 2 2 2 2 2 2 2 6 2 5 6	Date of 30.xii.51 per cent sev 2 37.6	Date of observation	31.i.52 st 27.2 100 (Almost dried up)	56	6.iii.52 67 (Harves- ted)	
) 1.5 4	4		32.2	62	91 (Withering)	100 (Dried)	:	:
0 T on 35% 2 plants	61		2.7	D on leaf sheaths; 2% on leaf blades	44	63	82 (Dried)	:
0 T on 5- T on 5-10° 10% plants	T on 5-1 plant	%0	T on 5-10% T on 50% plants plants		20.5 (D) on leaf sheaths. T	43	47	65
plants —do—	op		E ;	D on sheaths on blades	2.5 (D) on sheaths. T on blades	26	 .ç	. 20a
T	T on 10	%	T on 10%	T on sheaths.	D on sheaths	27	32	438
plants plants 0 — do— — do—	plants —do—		plants — do—		7 (D) on sheaths. T on blades	30	36	42a

*The date in brackets denotes the time of heading; T-Trace; D-The infection was developing when the observation was made. 10.xi.52, when a large quantity of spore material was also present in the field, infection appeared in varying intensities on different varieties. It was quite heavy on the green border plants of A.013 and Motia, although the plants away from the border, which matured earlier, were either free from infection or had only traces of infection. In A.115 and Niphad 4, infection did not exceed 10 per cent even on border plants. On N.P. wheats, it was noticed in traces when in dough stage. Only such plants as were free from infection or had only traces of infection were selected for calculating the weight of healthy grains, the details of which have already been given under 1950-51 trials.

The total yield of each plot was also recorded in this trial in order to obtain a rough idea about the yield under rust epidemic. The total yield, weight per 1,000 grains and the loss in grain weight, expressed as a percentage of the weight of an equal number of healthy grains are given in Table 4.

Table 4

The total yield, the weight of 1000 grains and the per cent loss in grain weight of eight wheat varieties grown under rust infection in 1951-52.

0 0							
Variety		R. I	R. II	R. III	R. IV	R. V	Mean
Niphad 4	a.	110.70	79.20	69.00	67.60	91.50	83.60
* (45.24)	b.	20.73	17.47	13.69	16.71	20.15	17.75
, ,	c.	54.20	61.16	69.74	63.06	55.46	60.72
Motia	a.	2.50	0.15	0.02	0.01	1.50	0.84
(53.56)	b.	3.30	1.74	2.00	2.00	3.63	2.53
•	c.	93.89	96.75	96.27	96.27	93.41	95.32
A.113	a.	1.08	0.15	0.01	0.025	0.19	0.29
(44.03)	b.	1.88	1.28	0.76	0.50	1.46	1.17
	e.	95.72	97.09	98.27	98.86	96.68	97.32
A.115	a. '	29.80	27.00	14.00	19.60	24.20	22.92
(41.65)	b.	6.52	6.02	4.81	5.00	5.69	5.60
,	c.	84.34	85.55	88.47	88.00	88.34	86.94
N.P.52	a.	173.70	158.2	141.20	154.60	194.20	164.38
(35.16)	Ъ.	24.06	25.14	23.97	24.98	22.22	24.07
· ·	c.	31.57	28.49	31.82	28.95	36.83	31.53
N.P. 114-3-2	a.	245.80	203.90	136.40	170.20	245.40	200.34
(40.63)	b.	36.23	35. 06	32.77	35.26	35.41	34.94
,	e.	10.83	13.71	19.35	13.22	12.85	13.99
N.P.710	a.	294.0	181.0	214.5	206.0	375.3	254.16
(41.03)	b.	37.7	35.55	37.26	34.57	37.4	36.45
	c.	8.11	13.35	9.19	15.74	8.85	11.05
N.P.715	a.	323.5	183.00	219.00	191.00	266.00	236.5
(42.52)	b.	38.4	37.61	38.12	37.99	38.28	38.08
,	c.	9.69	11.54	10.35	10.65	9.97	10.44

^{*} The figure in brackets denotes the mean wt. of 1,000 healthy grains in gms. (mean of five replications).

b. Wt. of 1,000 grains in gms.

a. Total yield in gms. obtained under rust infection.

c. Loss in grain weight expressed as a percentage of the weight of an equal number of healthy grains.

Analysis of variance

(Per cent loss in grain weight)

Factor	D. F.	Sum of squares	Variance	F.		
Total	. 39	52778.32				
Varieties	7	52429.86	7489.88	803.64 **		
Blocks	4	87.59	29.9			
Error	28	260.87	9.32			

Critical difference between any two variety means is 5.335 per cent (P=.01).

Conclusions

A.013, Motia, A.115, Niphad 4, N.P. 52, N.P.114-3-2, N.P.715, N.P.710.

It will be seen that the order of varieties according to the percent loss in grain weight is the same as in 1950-51. N.P. 114-3-2, N.P. 715 and N.P. 710, which were significantly different from one another in 1950-51, are not so in this trial. As in 1950-51, the grains of Motia, A.013 and A. 115 were very badly shrivelled. The development of grain of the last three N.P. varieties was quite satisfactory as will be seen from Plate I.

While harvesting the varieties in the rusted series, marked differences were noticed in the number of grains contained in the earheads of different varieties under trial. In Motia and A.013 there were, if at all, only 2 or 3 shrivelled grains in each earhead. In order to determine the effect of stem rust upon the number of grains per earhead, 40 earheads were selected at random from each replication and the number of grains in each was counted by threshing them carefully. The number of grains in each of the 40 earheads of rust-free plants, selected from each replication from the dusted series, was also found out. The average number of

grains per earhead of healthy and rust infected plants and the difference, expressed as a percentage of the former, are given in Table 5.

TABLE 5

Average number of grains per earhead in healthy and rust infected plants and the difference expressed as a percentage of the former.

Variety	*Average no. of grains per earhead in healthy plants (dusted series)	Aeverage no. of grains per earhead in the rusted series	Difference (Col. 2-3)	Difference expressed as percentage of Col. 2.
Niphad 4	32.97	25.88	7.09	21.50
Motia	32.40	2.34	30.6	92.80
A.013	26.81	1.80	25.01	93.29
A.115	38.26	19.28	18.28	41.70
N.P.52	36.24	31.61	4.63	13.00
N.P.114-3-2	41.98	33.65	8.33	19.85
N.P.710	37.27	30.33	6.94	18.60
N.P.715	36.64	31.57	5.07	13.83

^{*} Average of 200 earheads, 40 in each replication.

The effect of stem rust on grain setting is very high in A.013 and Motia and quite pronounced in A.115. A part of the differences between the number of grains in healthy and rust infected plants in all varieties is likely to be due to differences in soil fertility, since the dusted and rusted series were about 150 ft. apart. Anyway, it is clear that heavy infection at about the time of flowering results in poor setting of grains.

The results of the two years' trials are summarised in Table 6.

TABLE 6

Summary of the results of two years' trials of some improved wheat varieties under stem rust epidemic

	1950)-51	1951	1-52	
Variety	*Mean wt. of 1,000 grains in gms.	@Mean per cent loss in grain weight.	*Mean wt. of 1,000 grains in gms.	@Mean per cent loss in grain weight.	
1	2	3	4	5	
Niphad 4	12.29	72.76	17.75	60.72	
Motia	3.44	93.17	2.53	95.32	
Mondhya 3-2	5.98	86.21	E-rapidly		
A.013	2.10	95.20	1.17	97.32	
A.115	5.26	87.50	5.60	, 86.94	
N.P.52	19.64	46.76	24.07	31.53	
N.P.114-3-2	30.02	29.67	34.94	13.99	
N.P.710	34.28	20.31	36.45	11.05	
N.P.715	32.57	24.82	38.08	10.44	

^{*}Obtained under infection of stem rust.

Discussion

In the opinion of Macindoe (1931) the ultimate damage done by stem rust is largely determined by its effect on the quality of the grains produced. He also states that generally speaking, there is a relation between the amount or severity of rust present and grain quality but there also appears to be a tolerance to rust exhibited by some wheats, as, when other conditions are practically equal, some apparently susceptible wheats produce a more satisfactory grain sample than others.

It will be observed from Tables 1 and 3 that infection of stem rust appeared first on Motia and A.013 in both the trials and developed more rapidly than on other varieties. From the dates of heading given in those tables, it will be seen that the infection on these varieties at about

[@] Expressed as a percentage of the weight of an equal number of healthy grains.

the time of heading was much more than that on others. The mean percent severity of infection on them was above 40 percent at this stage in 1951-52 as against traces on N.P. 52, N. P. 114-3-2, N.P. 710, N.P. 715 and below 5 percent on Niphad 4 and A. 115. Further examination of these Tables will show that heavy infection developed subsequently on A.115, M.3-2 and Niphad 4 but not on the N.P. varieties. Unless infection experiments are carried out under controlled conditions at different stages of plant growth, it is difficult to explain the differences in the severity of infection of wheat varieties which are highly susceptible in the seedling stage to all or most of the races introduced in the epidemic.

Marked differences were, however, noticed in the infection of leaf blades of the different varieties. The leaf blades of Motia and A.013 were much more heavily infected than of others. Heavy infection on leaf blades as well as on leaf sheaths was responsible for withering of plants of Motia and A.013 at about the time of flowering whereas other varieties were apparently healthy at that time due to less amount of infection, particularly on the leaf blades.

Levine (1928) is of opinion that the susceptibility of plants does depend to a considerable extent on their stage of development and they seem to be quite susceptible in the seedling stage and again at about the heading stage, with a period of greater resistance in the jointing stage. Craigie (1945) has reviewed the results of several workers in this connection and there seems to be general agreement on this point.

During the course of testing different wheat varieties against stem rust under field epidemic at Mahableshwar, it has also been the general observation of the authors that nearly all the durum wheat varieties of Bombay, Madhya Pradesh, Hyderabad and Madhya Bharat are, as a whole, highly susceptible in all stages of growth. A large number of vulgare wheats, however, seem to be less susceptible than others of the same group in the preheading stage. Marked pre-heading resistance has been observed with Khapli, a dicoccum wheat, which is susceptible to race 42 and its biotypes. When sown in alternate rows with Vijay, a highly susceptible durum wheat, and both infected with races 42 and 42B when 3-4 weeks old, the infection on the former did not exceed 'trace' (only on the leaf blades), until about the flowering time, although the latter was heavily infected. Abundant infection was, however, observed on Khapli during the post-heading period. Similar pre-heading resistance has been observed with Kenya Governor, a vulgare wheat which is susceptible to race 21 in the seedling stage.

It appears from the observations on the severity of rust infection on different varieties at different periods that the leaf blades of Niphad 4, N.P. 52, N.P. 114-3-2, N.P. 710, and N.P. 715 possess considerable degree of resistance to stem rust and this is probably also true of A. 115 and Mondhya 3-2 to some extent. As has already been pointed out above, it would be necessary to carry out infection experiments under controlled conditions to find out whether this resistance holds good against all the races and under different temperature ranges.

It will be seen from the results in Tables 1 to 4 that N.P. 114-3-2, N.P. 710 & N.P. 715 withstood the epidemic of stem rust to a much

greater extent than other varieties. Since the type of infection present on them was of 'susceptible' class, they are classed as 'tolerant'. N.P. 52, though not as tolerant as these three N.P. wheats, is much superior to others in this respect.

The varieties included in the trials of both the seasons may be grouped into three broad groups on the basis of the severity of infection at different periods of growth and the ultimate loss in grain-weight:—

- (i) Highly susceptible in all stages of growth, the loss being almost 100 percent if abundant inoculum and favourable weather conditions prevail for about six weeks:—A.013 & Motia.
- (ii) Highly susceptible but apparently less susceptible between seedling and heading period than afterwards; leaf blades less susceptible than those of A. 013 and Motia. The infection does not develop as rapidly as on A. 013 and Motia and hence the damage would depend upon weather conditions and quantity of inoculum during the most vulnerable stage of the plant:—A. 115, Mondhya 3-2 and Niphad 4. The last one is less susceptible than the other two.
- (iii) Distinctly less severely rusted and less damaged in the field than varieties in groups (i) and (ii) under severe epidemic of rust (tolerant):—N.P. 114-3-2, N.P. 710, N.P 715 and N.P. 52. The last one is not as tolerant as the other three.

The results presented in Table 5 show that the effect of stem rust on the number of grains per earhead is quite pronounced in A.013, Motia and A.115 and appears to be influenced by the amount of infection present at about the time of flowering.

The yield in gms. per plot for 1951-52 is given in Table 4. Due to the small size of the plot and also due to presence of highly infected plants in A.013 and Motia in the dusted series, the data have not been analysed statistically. However, the results clearly point out the ability of N.P. wheats to withstand rust epidemic better than others. They also indicate that Niphad 4 is not as bad as A.115 but would rank below the N.P. wheats in point of tolerance to rust.

We shall now discuss the applications of the results obtained in these trials. In breeding rust resistant wheat varieties for Peninsular India, it has been observed that the chances of obtaining rust resistant and agronomically desirable lines are greater in a resistant x tolerant cross than in a resistant x susceptible one. Naturally, this is what one would expect. Trials of this nature would greatly assist the breeder in his choice of the susceptible parents for crossing purposes. The second application would be in pushing forward the cultivation of rust tolerant wheat varieties in preference to highly susceptible ones wherever possible as a means of reducing losses due to rust until such time when seed of highly rust resistant varieties is available in large quantities. The Bombay Department of Agriculture has, for example, recommended the cultivation of Niphad 4 (Kadam, 1944) as a rust tolerant variety and the cultivators are convinced about its superiority to other varieties such as Bansi, Baxi, Mondhya etc. in this respect. It, however, is sometimes

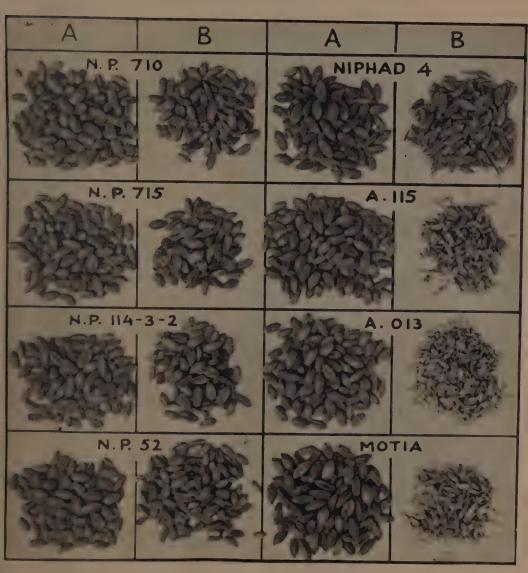


PLATE I 1951-52 Trials

A-Grains from healthy plants (dusted series).

B- Grams from plants under artificial epidemic of stem rust (rasted series).

badly damaged in areas under canal irrigation because of high humidity present in these areas almost throughout the growing period. been pointed out above, this variety possesses some pre-heading resistance but is likely to be badly damaged if weather conditions are favourable for rust infection in the post-heading period. The results of the trials carried out at Mahableshwar have indicated that N.P.710, N.P.715 and N.P.114-3-2 will fare better than Niphad 4 even in these areas. The third application of these results would be in the control of stem rust by sulphur dusting in years of heavy epidemic. It would be cheaper and more effective to adopt sulphur dusting as a measure of control with rust tolerant varieties than with highly susceptible ones. Growing rust tolerant varieties of wheat, coupled with a few dustings with sulphur, especially during the period from heading onwards, should prove a satisfactory and economical method of reducing the loss due to stem rust to a large extent in areas where highly resistant varieties are not available.

These results also indicate that the seedling reactions of rust susceptible wheat varieties, as determined in a glass-house, should not be solely relied upon for judging their behaviour under field conditions.

Yield is always the main consideration in the cultivation of wheat. Pal (1948) has shown that N.P.710 is a good yielder when tried at Karnal, Delhi, Nagina, Kanpur, Pusa and Kopargaon (Bombay) and that it was 'outstandingly good' at Kanpur. N.P.715 has done well at Kopargaon in both the years of trial. It will, therefore, be seen that in N.P.710 we have a wheat variety combining high yield and a high degree of tolerance to stem rust under epidemic conditions and should prove very useful for parts of Bombay, Uttar Pradesh and Bihar.

SUMMARY

The relative susceptibility of nine improved wheat varieties to stem rust, as measured mainly by the loss in grain weight, was studied in replicated trials at Mahableshwar, where an artificial epidemic of stem rust is created every year in the field, with ten Indian physiologic races. All varieties under test are highly susceptible to all or many of these races in the seedling stage. Two of them, viz. Motia and A.013 served as checks since they are observed to be heavily infected every year.

The varieties can be grouped into three broad groups on the basis of the severity of infection at different stages of growth:—(a) highly susceptible in all stages of growth, the development of infection being rapid; leaf blades highly susceptible:—A.013 and Motia; (b) highly susceptible but apparently less susceptible between seedling and heading stages than afterwards; leaf blades less susceptible than those of Motia and A.013:—A.115, Mondhya 3-2 and Niphad 4 (strictly speaking, Niphad 4 would be placed between this group and the next); (c) distinctly less severely rusted than varieties in groups (a) and (b) under an epidemic of rust (tolerant):—N.P.114-3-2, N.P.710, N.P.715 and N.P. 52. The last one is not as tolerant as the other three N.P. wheats.

The loss in grain weight did not exceed 30 percent in N.P.114-3-2, N.P.710 and N.P.715 and 50 percent in N.P.52 whereas it was almost 100 percent in Motia and A.013. A.115 and Mondhya 3-2 were almost

as badly damaged by rust as Motia and A.013 in these trials although they have been observed to be less severely rusted in some years when *Motia* and A. 013 are heavily infected. The loss in the grain weight of Niphad 4 was 72 percent in 1950-51 and 62 percent in 1951-52.

The effect of stem rust on the number of grains per earhead was studied only in one season. As compared to earheads obtained from healthy plants in sulphur dusted plots, the reduction in the number of grains was above 90 percent in A.013 and Motia and above 40 per cent in A.115. It ranged from 13 to 20 percent in other varieties.

Practical applications of these studies have been discussed. These are: (1) use of rust tolerant varieties in hybridization, (2) large scale cultivation of rust tolerant varieties where highly resistant varieties are not available and (3) application of sulphur dusting as a measure of controlling rust on rust tolerant varieties rather than on highly susceptible ones.

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DIPLOIDISATION IN UROMYCES HOBSONI VIZE AND PUCCINIA THWAITESII BERK.

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INTRODUCTION

Buller (1950) has recognized three periods of progress of cytological work on the initiation of the sexual process in the Uredinales. Period one starts in 1904 with the finding by Blackman (1904) of nuclear migrations from one cell to another in the aecial spore-bed of Phragmidium violaceum (Schultz) Wint. and ends with the discovery by Craigie (1927) of heterothallism in Puccinia helianthi Schw. The second period (1927-1933) witnessed the finding, again by Craigie (1933) of flexuous hyphae in the pycnia and their fusions with the pycniospores of opposite sex in *Puccinia* helianthi Schw. From 1933 onwards the third period commences. flexuous hyphae have, since then, been recorded by Pierson (1933) in Cronartium ribicola Fischer, by Hunter (1936) in eight species of Melampsoraceae, by Kamei (1940) in six species of Milesia, by Buller (1941) in thirty-one species of Uredinales, by Olive (1943) in Thekopsora hydrangeae (=Pucciniastrum hydrangeae) and by the writer (1952) in Scopella gentilis (Syd.) Mundk. & Thirum. Because of their widespread occurrence (known so far in 52 species of Uredinales) and because of their fusions with the pycniospores having been definitely demonstrated in Puccinia helianthi Schw, Cronartium ribicola Fischer, Puccinia coronata avenae F. & L., Puccinia graminis Pers. Gymnosporangium clavipes Cke, and Scopella gentilis (Syd.) Mundk. & Thirum. the 'Blackman type' of nuclear migrations and the 'Christman type' of cell-fusions have not retained the same importance and interest that they aroused at the time of their discovery. Even then until convincing evidence is obtained of how indeed the pycniospore nucleus migrates down the length of the flexuous hypha and how it initiates or stimulates the process of diploidisation, it seems best to continue to record the nuclear migrations or cell-fusions wherever they are encountered. Olive (1947) has recently shown the occurrence of nuclear migrations in the basidia of Sphenospora kevorkianii Linder which means that nuclear migrations are not necessarily confined to aecial or telial primordia. Wang and Martens (1939) in a comprehensive paper have attempted to solve the question of the origin of the dikaryophase in the Uredinales after studying the material of Puccinia caricis (Schum.) Rebent, P. coronata Corda, P. malvacearum Best, P. poarum Niels, and Uromyces poae Rabenh. The writer places on record in this paper his observations on nuclear migrations in Uromyces hobsoni Vize and cell-fusions in *Puccinia thwaitesii* Berk.

MATERIAL AND METHODS

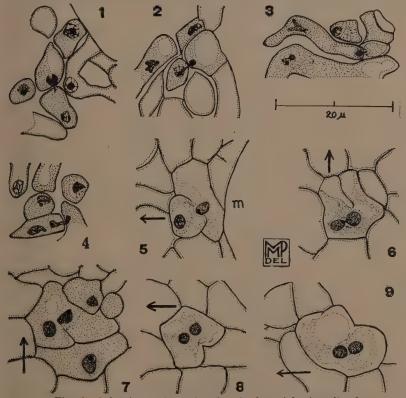
Young pycnial and aecial material of $Uromyces\ hobsoni$ was collected locally in Poona and fixed in Formol-Acetic-Alcohol. Microtome sections were cut to $8\ \mu$ thickness and stained in Heidenhain's haematoxylin and

Orange G in clove oil. Telial material of *Puccinia thwaitesii* was also gathered locally and fixed in Flemming's weak fluid. Sections 10 μ thick were cut and they were stained by Newton's Iodine-Gentian-Violet technique as well as in Heidenhain's haematoxylin. All figures were drawn at a magnification of X2300. They are here reproduced at X 1500.

UROMYCES HOBSONI

U. hobsoni is an opsis-form having repeating aecia in its life-cycle and is found on young leaves, twigs and flower buds of Jasminum grandiflorum L. Even though work has been done on its life-history and cytology by Ajrekar and Parandekar (1931) and by Thirumalachar (1939), the precise manner in which the binucleate condition in the aeciospores is achieved, had not been determined. Thirumalachar (1939) refers to occasional cell-fusions in the aecial primordia as a result of which some aeciospores became quadrinucleate because the rust found in Bangalore showed binucleate mycelium originating from binucleate basidiospores.

The nuclear migrations shown here (Figs. 1-4) were observed in the primordia of primary aecia. The aecial primordia in which diploidisation



Figs. 1—4 showing nuclear migrations in the acial primordia of *Uromyces hobsoni*. Figs. 5—9 showing cell-fusions in the telial primordia of *Puccinia thwaitesii* All X1500.

occurs have been given the separate name of 'proto-aecidia' by Buller (1938). The pycniospores and the pycniosporophores of the fungus available at Poona are uninucleate in contrast to the binucleate ones of the Bangalore fungus. The primary aecial primordia associated with such pycnia are composed of uninucleate cells throughout. Diploidisation by means of nuclear migrations has been found in those aecial primordia where the differentiation between the sterile peripheral layer which ultimately would have produced peridia and the inner fertile layer which would have finally given rise to aeciospores, had not yet occurred. The details given below refer to primary aecia associated with pycnia. Cytological details in the secondary aecia have not been investigated.

Fig. 1 represents an early stage of nuclear migration when only a small part of the nucleus in the form of a short spike has migrated in the adjacent cell below. In fig. 2 it appears as if the nucleolus is the last part to migrate, the rest of the chromatin having already gone earlier to the other cell. The absence of the original nucleus in the diploidised cell is explicable by assuming that some part of that cell has either gone in the next section, or has remained in the previous section. In fig. 3 major portion of the nucleus can be seen to have already migrated into the cell where another nucleus has assumed a peculiar forked appearance with its nucleolus located at the top of the right arm of the fork. Fig. 4 represents a case where the nucleus in the process of migration has not become as much attenuated as the migrating nuclei in figs. 1-3.

PUCCINIA THWAITESII

This is a micro-form rust without pycnia and commonly occurs on the leaves of the garden plant Justicia gendarussa L.f. in certain parts of the Bombay State. Its life-history has been worked out by Parandekar and Ajrekar (1932). Examination of microtome sections of material fixed early in the season of the appearance of the rust (September-October) revealed many cases of cell-fusions in the telial primordia (Figs. 5-9). Although it is known that in the telial fundaments of the same micro-rust both modes of diploidisation viz., cell fusions and nuclear migrations may occur, e.g. in Puccinia prostii Moug. worked out by Lamb (1934), in P. thwaitesii so far only the former type has been observed.

Diploidisation starts with the gradual dissolution of the septum between the two fusing cells. Though cases of nuclei being in a beaked condition while passing into the cells to be diploidised have been observed, no evidence of a septum between the cells was found. If the nuclei were to migrate either through intervening septal pores (Buller, 1950) or through perforated septa, they ought to have assumed the typical constricted form as shown here in *Uromyces hobsoni*.

The position of the fusing cells is variable in the telial primordia. In fig. 5 the fusing cells are lying parallel to the as yet unruptured lower leaf-epidermis indicated by an arrow on the left. These two fusing cells are located just at the base of the telial primordium on one side, as can be seen by their being adjacent to a cell \mathbf{m} of the leaf mesophyll. In fig. 6 the long axis of the diploidised cell is vertical to the leaf-epidermis shown by an arrow. The diploidised cell is particularly prominent in the telial primordium because of its being surrounded on all sides by highly-vacuolate

cells. The nuclei have come very near to each other. This means that fig. 6 represents a later stage than that shown in fig. 5. Diploidised cells in figs. 6 and 7 are similar except that in the latter the dissolution of the septum has started from above instead of from below. In figs. 7 and 8 the fusion cells are more or less inclined to one side of the leaf-epidermis again indicated by means of arrows. Figs. 8 and 9 represent later stages where almost complete absorption of the septa between the fusing cells has taken place. Note their bigger size in comparison with the smaller adjacent cells.

Discussion

The observation of constricted or attenuated nuclei while they migrate from one cell to another, according to Buller (1950), supports the existence of septal pores. He remarked "certain illustrations published by Rust cytologists suggest that stages in nuclear migrations through septal pores have actually been seen. As examples one may cite Figs. 3. 4, 5 and 6 on Plate III in Blackman and Fraser's account of the 'fertilisation' process in the proto-aecidium of *Puccinia poarum*". Parenthetically it may be mentioned that according to the legend of the figures given in the paper of Blackman and Fraser (1906) the nuclear migrations represented in the above-quoted figures were observed in *Uromyces poae* and not in *Puccinia poarum*, as Buller (1950) has wrongly stated.

As an instance, which does not support the contention of Buller purely on cytological grounds, may be cited figure 30 on Plate II in the paper on *Puccinia prostii* Moug. by Lamb (1934). Here the nucleus while going into another adjacent cell below has not become constricted, deformed, or beaked even though the intervening septum has been figured as intact. It appears as if no resistance has been offered by the septum which is highly unlikely. It also means that there did occur dissolution of the septum only to that much extent as that required for the migration of the nucleus in its normal form. The latter occurrence is more likely since in the same rust Lamb (1934) has shown cases of cell-fusions where the septa were partly absorbed. Fig. 37 in the paper of Olive (1908) depicting a case of cell-fusion in Phragmidium potentillae-canadensis Diet. is another Olive (1908) figures the existence of a "conjugation pore" not in the middle but on one side (left) of the septum. A discharged nucleolus has been shown passing through it. Here also, as in Puccinia prostii Moug., the septum might have been absorbed only partly. These examples point out the unreliability of conclusions about the existence of septal pores drawn from observations made on material fixed and stained by conventional methods. The microtome method does not permit us always to see the cells of the aecial primordia in their entirety in one view or one section and hence what may appear to be a central part of a cell may not be so in reality.

However, the observations made on the aecial material of *Uromyces hobsoni* do allow us to assume this much that the attenuated condition of the nuclei in their act of passing from one cell to another is primarily due to the resistance offered by the intervening septa. It is probable that the septa are porous all along and the labile nucleus passes only through that portion of the septum which offers least resistance. Further, it appears likely that the place of least resistance in the septum or the septum as a whole is just a sieve or wire-gauze-like region.

SUMMARY

- 1. An account of the 'Blackman type' of nuclear migrations in the aecial primordia of *Uromyces hobsoni* and the 'Christman type' of cell-fusions in *Puccinia thwaitesii* has been given.
- 2. It has been emphasized that from observations solely made on fixed and stained material, it is difficult to prove the existence of septal pores in the mycelial septa of any given rust fungus. It has been further suggested that the septa are probably wire-gauze-like and the nuclei migrate from one cell to another through that septal region which offers least resistance.

ACKNOWLEDGMENTS

The writer is grateful to Prof. S. L. Ajrekar and the late Dr. B. B. Mundkur for their encouragement and advice. He is also indebted to Dr. M. J. Thirumalachar for helpful suggestions. Part of this work was done in the Botany Laboratory of the Maharashtra Association for the Cultivation of Science, Poona. For the facilities provided there the writer expresses his grateful thanks to the Director, Dr. S. P. Agharkar.

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NOTES ON SOME FUNGI FROM SOUTH INDIA—I

T. S. RAMAKRISHNAN AND N. V. SUNDARAM

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During the course of tours to different parts of the State collections of fungi are made from time to time by the staff of the mycological section. Among these are some which have not been recorded from the State or from India or are new to science. It is proposed to issue annotated and descriptive notes of such fungi in this series. The specimens will be deposited in the Herbarium of the Government Mycologist at the Agricultural Research Institute, Coimbatore and in the Herb. Crypt. Ind. Orient. of the Indian Agricultural Research Institute, New Delhi.

Cystopus candidus (Pers.) Lev.

Saccardo, P. A. Syll. Fung., 7, 234, 1888.

On leaves of *Coronopus didyma* L. (Cruciferæ), Ootacamund, 27-10-52, T. S. Ramakrishnan and K. V. Srinivasan.

The host is a common weed on the Nilgiris. Heavy infection of this host was prevalent in and about Ootacamund. The white sori were conspicuous on the lower surface of the pinnules. Oospores were not present but the conidial measurements indicate the close identity of this fungus to *C. condidus*.

Empusa lecani Zimm.

Petch, T. Trans. Brit. Myc. Soc. 11, 254-258, 1926.

On *Phenacoccus insolitus* Green, infesting *Solanum melongena* L. (Solanaceæ), Coimbatore, 14-11-52, T. S. Muthukrishnan.

The fungus had covered the insects forming smoky grey growths. The conidia were ovate, light grey coloured and measured $19\times 9\mu$ ($12-22\times 7.5-12$). They readily germinated in water producing filiform germ tubes of limited growth bearing secondary conidia measuring $12\times 9\mu$ ($12-16\times 9-12$). Inside the body of the insects numerous hyaline, spherical bodies were present. These measured 12.4μ (9.3-15.5) in diameter. The characters of the fungus indicate that it is closely allied to *E. lecani*.

Phyllachora sorghi V. Hohn.

Saccardo, P. A., Syll. Fung., 22, 426, 1913.

On leaves of Sorghum vulgare Pers. (Gramineæ), Coimbatore, 2-1-53, N. V. Sundaram.

Heavy incidence of infection was prevalent in January on a number of varieties of cultivated sorghums on the Millets Breeding Station, Coimbatore and in the cultivators' fields in the vicinity. Several species

and varieties were involved. The incidence was observed on S. dochna var irungu (Burkill) Snowden, S. roxburghii Stapf, S. durra var. coimbatoricum (Burkill) Snowden, S. halepense, Pers., S. margaritiferum Stapf, S. elegans (Koern.) Snowden, S. conspicuum Snowden and a few other hybrid progenies.

Diorchidium levigatum Syd.

Sydow, H. and P. and Butler, E. J., Ann. Mycol., 5, 500, 1907.

On leaves of *Oplismenus compositus* Beauv. (Gramineæ), Walayar (Malabar), 16-11-52, N. V. Sundaram and C. P. Subramaniam.

This rust was first described by Sydow and Butler from Dehra Dun. The original description is about the telial state alone. The rust now recorded is the same fungus but has the uredial state also. The uredia are mainly epiphyllous with a few sori on the lower surface. They are formed in groups or arranged linearly in yellowish spots. They are subepidermal and erumpent. The urediospores are pedicellate, sub-globose or obovate, reddish brown, $28\times22\mu$ (19—40×15—25) with echinulate and coloured wall. There are four germpores arranged more or less equatorially.

The telia are black, erumpent and mainly hypophyllous. The majority of the spores have vertical or oblique partition walls and broad thickened apices. The pedicel is attached at the junction of the two cells or obliquely to one of the cells. Some spores are typical of *Puccinia* with the two cells placed one above the other. In such spores an apical thickening is present on the upper cell (up to 7.5μ). They measure $31\times22\mu$ (22—44×19—28). The spore wall is thin and the pedicel is hyaline measuring up to 78μ and gelatinising in water. Germination takes place without any rest and the promycelia are long.

Puccinia operculinæ sp. nov.

Uredia amphigenous, subepidermal, erumpent, chocolate brown, numerous; urediospores pedicellate, subglobose to oblong, dark brown, finely verrucose, wall coloured, 3-4 pores on the upper half of the spores, $22\times19\mu(19-28\times12-22)$; telia rare, mostly on the stem, long covered by the epidermis, isolated or confluent, bounded by closely packed incurved paraphyses, bigger telia divided into compartments by groups of vertically arranged brown paraphyses; teliospores stalked, with brown pedicel up to 53μ long, elliptical or oblong, $47\times19\mu$ ($40-62\times16-25$), apex flattened, sometimes tapering, thickened up to 12μ , constricted at the septum; mesospores rare, $27-38\times15-19\mu$.

Uredia amphigena, subepidermalia, erumpentia, castaneo-brunnea, plura; uredosporæ pedicellatæ, subglobosæ vel oblongæ, fusce brunneæ, minute verrucosæ, parietibus coloratis, 3-4 poris ornatæ in superiore sporarum dimidio, $22\times19\mu$ (19—28×12—22); telia rara, ut plurimum in caule, longe operta epidermide, distincta vel confluentia, circumdata paraphysibus incurvis dense aggregatis, telia largiora divisa in sectiones per paraphyses brunneas, verticaliter dispositas; teliosporæ pedicellatæ pedicello brunneo usque ad 53μ . longo, elliptieæ vel oblongæ, $47\times19\mu$ (40—62×16—25), apice complanato, aliquando fastigatæ, incrassatæ usque ad 12μ , constricatæ ad septum; mesosporæ raræ, 27—38×15—19 μ

On leaves and stem of *Operculina turpethum* S. Manso. (Convolvulaceæ), Walayar (Malabar), 16-11-52, N.V. Sundaram and C. P. Subramaniam.

Urediosori are predominant. The telia are rare and often confined to the stem. The characteristic division of the telia into compartments by groups of brown septate paraphyses is peculiar and differentiates this from other species occurring on *Ipomoea*.

Puccinia phragmitis (Schum.) Koern.

Saccardo, P. A. Syll. Fung., 7, 630, 1888.

On leaves and leaf sheath of *Phragmites karka* Trin. (Gramineæ), Walayar (Malabar), 16-11-52, N. V. Sundaram and C. P. Subramaniam.

Uredia and telia are present on the leaves and leaf sheaths. The urediospores are long stalked, elliptic, obovate or oblong with four equatorial germpores and coloured verrucose wall. Paraphyses are absent. Telia are blackish brown, erumpent and elongated. The teliospores are elliptic, smooth-walled and constricted at the septum. They are formed in clusters borne on long, flexuous, subhyaline pedicels up to 210μ long.

P. invenusta. Syd. has been described on this host from India. But the rust under study resembles P. phragmitis. In the former, clavate praphyses have been observed in the uredia and the stalk of the teliospore is brown and up to 60μ long. On account of these differences the rust is identified as P. phragmitis. The teliospores germinate readily without a rest period. Polyganum chinense L. has been found to serve as an alternate host for this rust. Infection experiments with germinating teliospores of P. phragmitis have resulted in the production of pyenia and aecia on P. chinense. The aecia developed in the course of P. weeks.

Ravenelia clemensæ Syd.

Sydow, H. and Petrak, F. Ann. Mycol., 26, 418, 1928.

. On living leaflets of *Albizzia procera* (Roxb.) Benth., (Mimosoideæ) Walayar (Malabar), 14-12-52 N. V. Sundaram.

The sori are amphigenous on the leaflets. The uredial and telial characters indicate that the rust is $R.clemens \alpha$. This has not been recorded from India.

Ravenelia hansfordii Cumm.

Cummins, G. B. Bull. Torrey Bot. Club, 72, 214, 1945.

On leaflets of Acacia suma Buch-Ham. (Mimosoideæ), Walayar (Malabar), 16-11-52, N. V. Sundaram and C. P. Subramaniam.

The rust sori are common on the leaflets. The uredia are amphigenous but more on the upper surface. Uniseptate, clavate or cylindric paraphyses are produced in large numbers all along the margin of the sori. The urediospores are spindle shaped with about four equatorial germpores. The telia are black and the spore heads are

chestnut brown with 5-7 spores in cross section and possess few hyaline wart like projections. Teliospores are formed in uredia also. The cysts are hyaline and pendulous. The rust resembles closely R. hansfordii. R. acaciæ-sumæ Mund. and Thirum. has been recorded on this host from Mysore. But the bigger size of the urediospores and the characteristic paraphyses indicate that the rust under study is different from it.

Révenelia hansfordii Cumm. var. ferrugineæ var. nov.

Uredia amphigenous, subepidermal, praphyses numerous, cylindrical sometimes one septate, subhyaline, $28-43\times6-12\mu$; urediospores elliptical, $22\times12\mu$ (13—31×9—16), with 4 equatorial germ pores; telia amphigenous, subepidermal, blackish brown, teliospore head convex, chestnut brown, 66μ (49—79) in diam., spores one celled; verruciform papillæ; many pendulous, globose, hyaline cysts.

Uredia amphigena, subepidermalia; paraphyses plures, cylindricæ, nonnumquam semel septatæ, subhyalinæ, $28-43\times6-12\mu$; uredosporæ ellipticæ, $22\times12\mu$ ($13-31\times9-16$), ornatæ 4 poris germinationis equatorialibus; telia amphigena, subepidermalia, fusce brunnea; teliosporarum capita convexa, castaneo-brunnea, 66μ (49-79) diam., sporæ unicellulatæ; papillæ verruciformes; cystes plures, pendulæ, globosæ hyalinæ.

On living leaves of *Acacia ferruginea* DC. (Mimosoideæ), Walayar (Malabar), 16-11-52, N. V. Sundaram and C. P. Subramaniam.

The uredia are amphigenous with marginal cylindric paraphyses as in the case of *R. hansfordii*. The urediospores are elliptic, with 4 equatorial germpores. The telia are also amphigenous and the teliospores resemble those of *R. hansfordii*. This rust resembles the former in many respects except in the size and the shape of the urediospores and is therefore described as a new variety.

Pestalotia seiridioides Sacc.

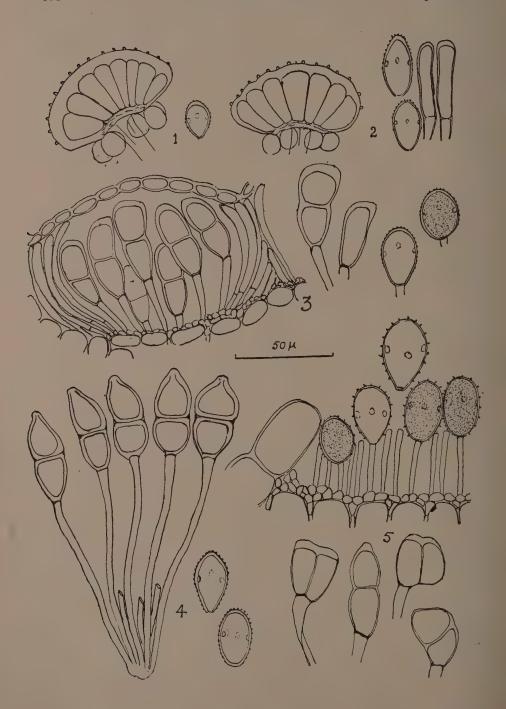
Saccardo, P. A. Syll. Fung., 3, 799, 1884.

On leaves of Rosa leschenaultiana W. & A. (Rosaceæ), Ootacamund, 27-10-52, T. S. Ramakrishnan and K. V. Srinivasan.

Brown indefinite spots with grey centres were found on the leaflets. The acervuli were present in the grey portions on both sides of the leaves as black, erumpent, disciform, elongate bodies. The conidia are five septate with four central coloured cells and one conical hyaline cell at each end. Usually a single hyaline cilium was present but rarely the cilia gave rise to 2 or 3 branches at the apex. Though the fungus has been noticed on the stem in other countries, in the specimens now described the acervuli are found in the affected portion of the leaflets.

We are indebted to Rev. Dr. H. Santhapau for the latin translation.

Agricultural College Coimbatore.



ILLUSTRATIONS

- Fig. 1. Ravenelia hansfordii var ferrugineæ, Teliospore head and urediospore.
 - ,, 2. R. hansfordii. Teliospore head, urediospores and paraphyses.
 - ,, 3. Puccinia operculinæ Telium, teliospore, mesospore and urediospores.
 - " 4. P. phragmitis. A cluster of teliospores and urediospores.
 - ,, 5. Diorchidium levigatum. Uredium and teliospores.

SOME PHYTOPATHOGENIC BACTERIA REQUIRING REINVESTIGATION

M.K.PATEL, V.V.BHATT and Y.S.KULKARNI

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Elliott (1951) records 301 bacterial plant pathogens so far described of which only 195 are claimed to be true pathogens, the rest being considered doubtful since their pathogenicity is uncertain. Since the time of E.F. Smith, a number of workers have studied this group and published voluminous literature mainly relying upon the work of Bergey et al., Burkholder, Dowson, Elliott etc. Definite reactions of an organism as symptomatical, morphological, cultural and bio-chemical behaviours have now been established as a result of which the organism under consideration can be placed in a definite genus. Thus, motility, gram reaction, pigmentation, carbon metabolism, pathogenicity and symptoms on hosts are considered of fundamental importance in assigning a proper nomenclature to the organism. Different classifications are suggested by workers and attempts made to accommodate the plant pathogenic bacteria in a genus according to their early descriptions. In these attempts, most of the workers mainly depended upon the original description and changed the generic position of the organism from one to another taking one or two characters into consideration. It is also observed that a number of species are not fully described and naturally it becomes difficult to assign them a proper taxonomic position. Although there exist no rules for a definite description of a new organism, this science is now very well advanced and workers know the importance of each test. In classification, chief characteristics of a genus are given by the taxonomists which should be observed while assigning a definite status to an organism covering all the characters of that genus. For instance, Xanthomonas, according to Dowson (1949), is yellow pigmented, uniflagellate, gram negative, producing mostly leaf-spots, acid without gas from lactose and no acid from salicin. It will be out of place to assign an organism having gram positive reaction or producing gas from carbohydrates or whitish in colour to Xanthomonas. this paper, the writers propose to point out such irregularities as a result of which the taxonomy of such organisms becomes doubtful and a general request is, therefore, made to workers having facilities and such cultures to study them so as to remove discrepancies. Patel and Kulkarni (1951) have suggested a nomenclature with definite characters for a genus and the following observations are based upon it. However, Elliott's classification (1951) is followed here to point out the existing discrepancies.

CORYNEBACTERIUM

Of all the genera of the phytopathogenic bacteria, Corynebacterium (Aplanobacter) alone includes non-motile and gram positive organisms besides being pathogenic to plants. Thus, C. agropyri (O'Gara) Burkholder, C. rathayi (Smith) Dowson and C. tritici (Hutchinson) Bergey et al. which have been stated to be non-pathogenic as a result of unsuccessful artificial inoculations by O'Gara (1916), Dowson and D'Oliveira (1935), Cheo (1946)

and Vasudeva and Hingorani (1952) respectively should, therefore, not even find a place in Phytobacteriaceae.

C. agropyri is reported as gram negative while Bergey et al. (1948) report the same as gram variable. C. poinsettiae Starr and Pirone (1942) is reported as gram variable, a vague term to be avoided for studies and therefore the organisms should not find a place in this group. C. hypertrophicans (Stahel) Burkholder and C. poinsettiae have been reported to be motile and therefore should be dropped from this group. The thermal death point of the phytopathogenic bacteria generally falls around 50°C., whereas C. flaccumfaciens (Hedges) Dowson, having the thermal death point of 60° C., creates reasonable doubt of its being a mixed culture when studied.

ERWINIA

The genus Erwinia of Bergev et al. (1948) now includes Pectobacterium and Erwinia according to Patel and Kulkarni (1951) representing gram negative and peritrichiate organisms. Hence E. citrimaculans (Doidge) Magrou, E. lilii (Uyeda) Magrou and E. mangiferae (Doidge) Bergey et al. if gram positive and E. dissolvens (Rosen) Burkholder motile by one polar flagellum or non-motile should not be included in this group. The genus is further characterised by white or dirty white pigment and hence E. ananas Serrano, E. cassavae (Hansford) Burkholder, E. erivanensis (Kalantarian) Bergey et al., E. flavida (Fawcett) Magrou, E. salicis (Day) Chester and E. vitivora (Baccarini) DuPlessis with yellow pigment should have no place in this genus. The optimum temperature for growth of the phytopathogenic bacteria generally lies between 27° and 30°C. whereas E. citrimaculans, E. ananas, E. aroideae (Townsend) Holland, E. betivora (Takimoto) Magrou and E. lilii with 35°C. as optimum temperature lead to some doubt. Similary, the thermal death point of E. citrimaculans, E. ananas, E. carnegieana Lightle, Standring and Brown and E. mangiferae is reported to be above 57°C., while that of E. tracheiphila (Smith) Holland is as low as 43°C, and E. nimipressuralis Carter with a range of 45° to 55°C. also points to study with impure cultures.

Bergey et al. (1948) consider E. flavida as a chromogenic strain of E. carotovora (Jones) Holland and unless cross inoculations are made, nothing can be said as organisms in the same genus may have similar cultural and bio-chemical reactions. In a natural way of classification, symptoms do play an important role as has been indicated by Patel and Kulkarni (1951). Thus Erwinia produces blight and Pectobacterium softrot only while E. milletiae (Kawakami and Yoshida) Magrou produces knots and galls on the stem, symptoms typical of the genus Agrobacterium. Moreover, as E. milletiae produces waxy yellow colonies on agar medium, its inclusion in the genus Erwinia may not be proper.

PSEUDOMONAS

Pseudomonas of Bergey et al. (1948) now includes Phytobacterium and Chlorobacter according to Patel and Kulkarni (1951) with normally white and green fluorescence respectively, gram negative, with 2 to 7 polar flagella and producing mostly leaf-spots. Thus Ps. cissicola (Takimoto) Burkholder, Ps. radiciperda (Javoronkova) Savulescu and Ps. rhizoctonia

(Thomas) Stevens being non-motile and Ps. mors-prunorum Wormald, Ps. polygoni (Thornberry and Anderson) Burkholder, Ps. seminum Cayley and Ps. viciae Uyeda being gram positive as reported should find no place in this genus. It is also stated that Ps. marginalis (Brown) Stevens is identical to Bacillus pyocyaneus, Ps. polycolor Clara to Ps. aeruginosa, Ps. rumaefaciens Koning to Ps. syringae van Hall and Ps. desaiana (Burkholder) Savulescu to Ps. lapsa (Ark) Starr and Burkholder. Besides, B. pyocyaneus and Ps. desaiana are definitely saprophytic cultures and, even though culturally and bio-chemically same, should not be accommodated here. From the symptomatical point of view, Ps. ananas and E. ananas, both causing fruitlet black-rot of pineapple, seem to be synonyms. But unless cross inoculations are tried and detailed cultural and bio-chemical tests made, it is futile to opine. The optimum temperature for growth of Ps. alboprecipitans Rosen, Ps. fabae (Yu) Burkholder, Ps. iridicola (Takimoto) Stapp, Ps. panici-miliacei (Ikata and Yamauchi) Savulescu as reported is 35°C. or more, while that of Ps. barkeri (Berridge) Clara, Ps. colurnae (Thornberry and Anderson) Burkholder, Ps. polygoni and Ps. primulae (Ark and Gardner) Starr and Burkholder at 20°C. or below. The thermal death point of Ps. alboprecipitans, Ps. bowlesiae (Lewis and Watson) Dowson, Ps. glycinae var. japonica (Takimoto) Savulescu and Ps. nectarophila (Doidge) Rosen and Bleeker is reported at 40°C. or below, while that of Ps. mellea Johnson, Ps. petasitis (Takimoto) Savulescu and Ps. setariae (Okabe) Savulescu at 55°C. or above. All this requires reinvestigation.

XANTHOMONAS

The genus Xanothomonas is generally accepted as yellow pigmented, gram negative, motile by single polar flagellum, producing mostly leafspots, strong acid in lactose but not in salicin. Therefore, 'X. conjaci (Uyeda) Burkholder and X. proteamaculans (Paine and Stansfield) Burkholder being gram positive and X. cannae (Bryan) Savulescu, X. manihotis (Arthaud-Berthet) Starr, X. panici (Elliott) Savulescu, X. rubrisubalbicans (Christopher and Edgerton) Savulescu, X. solanacearum (Smith) Dowson and X. zingiberi (Uyeda) Savulescu producing white to dirty white pigment should not be included in this genus. Also X. cannae, X. lactucae-scariolae (Thornberry and Anderson) Savulescu, X. lespedezae (Ayers et al.) Starr, X. panici, X. plantaginis (Thornberry and Anderson) Burkholder and X. rubrisubalbicans should find no place here since full reports on carbon metabolism are not made. Also X. holcicola (Elliott) Starr and Burkholder and X. manihotis not fermenting lactose, X. heterocea (Vsorov) Savulescu fermenting salicin but not lactose and X. conjaci and X. proteamaculans producing gas require a thorough reinvestigation as regards carbon metabolism. Cross inoculation trials for X. incanae (Kendrick and Baker) Starr and Weiss and X. itoana (Tochinai) Dowson reported to be identical in cultural and bio-chemical tests to X. campestris (Pammel) Dowson and X. oryzae (Uyeda and Ishiyama) Dowson respectively should be carried out.

It is also felt that cross inoculations should be carried out for X. rubrilineans (Lee et al.) Starr and Burkholder, X. albibineans (Ashby) Dowson, X. vasculorum (Cobb) Dowson and X. rubrisubalbicans as there exists some confusion. X. beticola (Smith, Brown, Townsend) Savulescu produces nodular to large, rough, fissured galls on beets, X. incanae and

X. manihotis attack primarily the vascular system and in X. solanacearum the natural infection takes place through the tubers. As X. beticola produces galls, it maybe included in Agrobacterium while the rest require reinoculation trials on leaves to see the mode of entry and production of wilt.

From this laboratory, Patel et al. have described about 32 bacterial plant pathogens of which some are already reported in other countries. Thus Xanthomonas alfalfæ (Riker, Jones, Davis) Dowson, X. begoniæ (Takimoto) Dowson, X. campestris (Pammel) Dowson, X. citri (Hasse) Dowson, X. malvacearum (Smith) Dowson, X. phaseoli var. sojense (Hedges) Starr and Burkholder, X. ricinicola (Elliott) Dowson, X. vesicatoria (Doidge) Dowson, X. vignicola Burkholder and Phytobacterium solanacearum (Smith) Patel, Kulkarni and Kulkarni are found to be identical in most of the important characters given by other workers for the above organisms. While for the following organisms, writers believe that a thorough reinvestigation is necessary in order to prove their synonymy with those reported by Patel et al. South Africa, Doidge reported Erwinia mangiferæ (Doidge) Bergey et al. on Mangifera indica also parasitised by Phytobacterium mangiferæ-indicæ (Patel, Moniz and Kulkarni) Patel and Kulkarni in India. Both the organisms are closely allied to each other in all important characters except motility. The same is true for Pseudomonas betlis (Raghunathan) Burkholder causing primarily a leaf-spot of *Piper betle* on which *Xanthomonas betlicola* Patel, Kulkarni and Dhande is also described. Xanthomonas stizolobiicola Patel, Kulkarni and Dhande is closely allied to Pseudomonas stizolobii (Wolf) Stapp, both causing leaf-spot on Stizolobium deeringianum. As regards Xanthomonas poinsettiaecola Bhatt and Kulkarni, the resemblance to Corynebacterium poinsettiæ Starr and Pirone (1942) is doubtful as the description is very vague.

BACTERIUM

The present status of the genus *Bacterium* can be compared with "Fungi imperfecti" which is in existance due to lack of full knowledge. Of the 5 species of *Bacterium* reported by Elliott (1951), *B. stewartii* Smith is worked out extensively. Yet its inclusion in this genus is regrettable. All require thorough investigation.

Hosts like Nicotiana tabacum, Phaseolus vulgaris, Saccharum officinarum, Solanum tuberosum, Sorghum vulgare, Zea mays etc., have been parasitised by many species either of the same genus or different, producing same or different symptoms. Moreover, some of the pathogenic species have very large host range some of which are attacked by other species. The strains of the same organism may show slight variation in its pathogenicity and bio-chemical behaviours and thus it is thought worthwhile to undertake study of such organisms at the same time and under the same environment.

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STUDY OF PHYSIOLOGIC RACES IN LINSEED RUST [MELAMPSORA LINI (PERS) LEV.]

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Introduction

Linseed rust caused by *Melampsora lini* (Pers) Lev. has long been known in India as a parasite attacking cultivated linseed *Linum usitatissimum* L. more commonly known in western countries as flax with which it bears certain morphological differences and grown in India mainly for the oil which the seed contains.

Butler (1914) recorded its occurrence on linseed at Pusa as early as 1914, indicating its menace as an epidemic throughout the country. Though he did not observe rust on flax at Pusa, Bihar, where it was grown for several years in the neighbourhood of severely infected linseed, he reported its occurrence on flax grown in the experimental plots in Nilgiris and indicated the possibility of extreme specialisation basing his conclusions on the fact that the two crops are merely cultivated races of the same plant. The rust has also been reported by other workers from different parts of the country where the linseed is grown.

Under epidemic conditions, the rust does considerable damage to both fibre and seed production, lowering the quality and quantity of both, sometimes entirely destroying their market value. The fibre is rendered brittle and useless. The loss of seed is reported to the extent of about 28 per cent by weight. But it is mainly for the seed production that the present extension of linseed cultivation is taking place in this country, which is its second biggest producer after Argentine*. Numerous reports of rust damage in indigenous varieties which are otherwise suitable for seed production, indicate that a programme for breeding for rust resistance will have to be carried out. It has long been established that inheritance of rust resistance in *Linum usitatissimum* L. is a genitical problem and that the resistance is inherited as dominant character. The control of rust lies largely in the breeding of rust resistant strains has been shown by Bolley (1906) and Henry (1926).

As has already been mentioned the linseed plant in India is grown entirely for the oilseed and not for the production of fibre and subsequently research work has been directed toward improving the quality and yield of oilseed. Preliminary efforts toward breeding strains that could combine high oil-content, early maturity and certain other desireable characters of I. P. types, constituting an all-India collection of indigenous linseeds and rust resistance of certain Australian varieties, have already been made in the Botany Division in co-operation with the Mycology Division of this Institute (Deshpande, 1950). As a result of this work 150 promising cultures were isolated and 72 of them were

^{*} Report of the Marketing of Linseed in India (I.C.A.R.), 1938

tested for rust resistance under conditions of artificial infection with rusts obtained from different linseed growing tracts of India. The results obtained are encouraging and have also indicated the possibility of the existance in India of several physiologic races of the rust (Prasada, 1948). It needs no special mention that for ensuring work in this direction a success, an organised effort on a sound basis is very essential. And toward such an effort, importance of the knowledge of the physiological specialisation shown by the pathogen need not be stressed.

Rust fungi show extreme specialisation. Thus, in the wheat stem-rust fungus about 200 physiological races have been determined. Studies on the rust of flax with reference to its specialisation are comparatively recent. The work in this direction has extensively been done in the U.S. A. by Flor (1935), who till 1940 recorded 24 races of this rust (Flor, 1940). In Australia, Waterhouse (1943) recorded till 1943 six races different from those reported by Flor (1940). Straib (1939) has carried out work in Germany on the host-range aggresiveness of the physiologic races of the fungus and has reported occurrence of eight races from Germany and the low countries of Europe. Vallega (1942) has done extensive study of the rust in South America and till 1942 reported occurrence of races 42 and 42 A, among others, from Argentine. The results of their work show that the race populations of United States of America, Australia, Germany and Argentine are entirely different.

Preliminary attempts toward determination of Physiological specialisation of the rust have already been made in this country. Padwick (1940) used spores from two seperate collections from Pusa and Karnal for inoculation of indigenous linseed type Pusa 21 and exotic flax variety J. W. S. His results point to the existance of at least two physiological races of linseed rust in India. Prasada (1948) indicated presence of five physiologic races by preliminary tests made on fifty varieties of linseed and flax, though he did not use varieties used by Flor (1940).

EXPERIMENTAL

· In the work under review, in all 25 rust collections from all over the country from the crops of 1945-46 and 1946-47 have been studied. The samples came from cultivated varieties of linseed growing in the following localities:—

I.	Punjab—Arki & Dhami(Simla hills), Sohawra (Kangra valley), Karnal, Ludhiana,		
	Jullundar and Gurdaspur	• • •	7 Collections
II.	Delhi—New Delhi	1	1 Collection
III.	UTTAR PRADESH—Basti, Kanpur, Allahabad		
	and Banda	•••	6 collections
IV.	Bihar—Pusa and Sabour	•••	4 collections
V.	CENTRAL INDIA-Gwalior, Ujjain and		
	Chattarpur State	•••	3 collections
VI.	Madhya Pradesh—Hatta, Saugar, Piparia		
	and Chaurai		4 collections

The technique adopted by Flor (1935) has been followed excepting that no provision was made for a constant light-day of 16 hours. Normal hours of day light at Simla (7,000 ft. a.s.l.) have been used and observations have been carried through all the seasons of the year. In addition to the seed of the eleven rust differential varieties used by Flor (1940)*, the following varieties were used **:—1. Argentine C. I. 705-1, 2. Bison and 3. Punjab C. I. 20.

As will be evident later, one of these varieties (Bison) has been found indispensible in the determination of physiologic race flora of this country.

It is well-known that environmental conditions, and particularly variations in temperature and humidity cause changes in the rust reactions exhibited by particular differential host; and this becomes an important consideration in the determination of physiologic races. (1935) reports that in his investigations he found that certain of his differential varieties were sensitive to such changes. One of them was Williston Brown. Waterhouse (1941) found the same true in case of Akmolinsk. In present studies varieties Williston Golden, Akmolinsk, J. W. S., & Argentine C. I. 705-1 were found to show a slight degree of reactional variation due to such changes of environment. Dry and cold weather accompanied by bright sunlight has been found to favour a higher stage of infection as compared to damp and hot weather accompanied by diffused sunlight. Variation in case of Argentine C. I. 705-1 has been remarkable in that the former set of environmental conditions have invariably been found to favour resistance (R) and the latter immunity (i).

Flor (1940) states that "attempts to differentiate too finely between degrees of resistance and susceptibility may lead to confusion and to misunderstanding of results obtained at different localities or under variable conditions". Keeping this in view, minor differences shown in race determination under review here have been eliminated and as such, it is considered that 18 collections out of 25 studied, may be regarded as falling within one physiologic race provisionally designated as I_1 , the other three races have also provisionally been designated as I_2 , I_3 , and I_4 . All the four races are new and have not been reported anywhere else.

The reactions of differential host varieties to single spore cultures of the four Indian races, provisionally designated as I_1 , I_2 , I_3 , and I_4 are given in Table I.

^{*} The list of differential varieties has since been revised and expanded by him. There are now 16 main and 5 auxilliary varieties. Seperate identity of Indian races, however, remains unchanged in relation to new set and will form the basis of another publication.

^{**} The seed was obtained through the kind courtsey of Prof. W. L. Waterhouse of the University of Sydney, Australia.

TABLE I

Reactions of Indian races of Melampsora Lini on standard differential varieties.

Lyallpur local	+ 22	+ 22	+ 2	~ +
Punjab	<u>ω</u>	202	ω	Ω2
Bison (American Variety)	•=	or	Z	•
Argentine I.307, 705-1	(i)	7. 5 t	4 ö f	+ 1 5 M
Bombay	202	Ω	<u></u> 20	<u>~~~~</u>
Argentine C.I. 462	•=	٠,	• (**)	·m
Ottawa C.I. 770 B	٠,	٠,	•	•=
Pale Blue Crimped C.I. 647-1	•=	•=	•=	· H
Williston Brown C.I. 803-1	R-	M	- R	H H
Kenya C.I. 709-1	•=	٠.	, - =	•=
Abyssinian IO, T. 701	٠,	•	•	•
J.W.S. C.L. 708-1	A Or	SR R	(R)	SR SR
Akmolinsk C.I. 515-1	SR	S.R.	SR	S S S S S S S S S S S S S S S S S S S
Williston Golden C.I. 25-1	M	24	SR	i or R+
Buda C.I. 270		i	M: 5	SR SR - S
Stock Collection to roitslosi	Stock	Stock	Buda (R)	Stock
tsoH laniginO	Local	Local	Hybrid C 4-2	Local
Locality	SOHAWRA	SABOUR	LUDHIANA	JULLUNDER
Proposed Race nomenclature	l I	I ₂	25	T ₄

i=immune, R=Resistant, SR=Semi-resistant, S=Susceptible.

NOTE. -Minus sign indicates romewhat less resistance or susceptibility & plus sign somewhat higher resistance or susceptibility than shown by letters designating normal host reactions.

DISCUSSION

A comparison of these results with those recorded by Flor (1940) shows that all the four races recorded here are different from any listed by him. Confirmatory evidence is available from two sources. In the first place, variety Bombay has been very highly susceptible in all our tests which is so to only race 24 recorded by him, to which unlike any of the four races recorded here, variety Pale Blue Crimped is susceptible. Again, variety Kenya is uniformly immune in all our tests, which is so to only race 11 listed by him; and to which again, unlike any of the four races recorded here, variety Williston Brown is susceptible.

A comparison of these results with those obtained by Waterhouse et al (1943) shows, however, that there is a great similarity of race I₁ to his race A and race I4 to his race F, though it is strikingly apparent that variety J.W.S., which is immune to races both A and F, is resistant or semi-resistant to Indian races I₁ and I₄, but never immune. In fact, this variety has never been immune to any of the Indian races or rust collections so far studied. As pointed out by him, race F shows affinities with race A, but is to be clearly separated from it by its reaction on Buda and it is apparent from the table that the same also applies to Indian races I₄ and I₁, though the difference is not so well marked as in case of the two Australian races referred to above. He observes that race A is particularly virulent on Indian linseed: varieties like Punjab and Bombay are very heavily attacked by it. He also considers this rust strain as essentially of linseed as differentiated from that of flax, and causing a serious damage on the former. His observations justly apply to Indian race I₁, to which as is shown, it bears striking resemblance, and when it is remembered that this particular race was met with in 18 out of 25 collections analysed from all the six regional localities under study. The Australian race F, which bears resemblance to Indian race I₄, has been found only on *Linum marginale*, a wild flax (Waterhouse *et al*, 1943). The Indian race, however, was picked up from cultivated varieties of linseed (Linum usitatissimum L.).

The race I_2 is differentiated from all other races by the reaction of Bison, which is infected by it. It is a rare race being picked up only once. Judged by the reactions of eleven standard differential varieties used by other workers reference to which has already been made, races I_2 and I_3 appear to be identical, except that the latter is more virulent. But the use of variety Bison, which is not listed as a standard differential, has been able to differentiate the two Indian races. The reaction of Buda to race I_3 is, however, variable; and under extreme conditions of weather, this race has been found to behave, more or less, like race I_1 , Buda showing immunity.

A striking feature of all Indian races of the linseed rust so far isolated is that, whereas varieties Abyssinian, Kenya, Pale Blue Crimped, Ottawa and Argentine C.I. 462 are uniformly immune, the varieties Bombay and Punjab (un-listed) are uniformly susceptible. This has limited the range of host differentials, particularly when it is observed that the races show but little differential responses on other varieties, namely, Williston Golden, Akmolinsk, J.W.S. and Williston Brown. This emphasises earlier remark that varieties not regularly listed as Bison, need to be included for determination of phyiologic race flora of this country and that there is need of extending or supplementing existing host range

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suitably by further trials on indegenous, as well as exotic varieties of linseed. Use of auxilliary differential varieties determined by suitable trials will, thus, ensure isolation of races properly, and which would be indispensible for testing, should intensive work on breeding of linseeds be undertaken in near future.

The results obtained here lend further support to the view that the race populations of the rust under review of different countries differ widely and that within each geographic population the races possess common characters indicative of their origin.

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NEW PHYSIOLOGIC RACES OF WHEAT RUSTS IN INDIA

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The study of physiologic races is an important aspect of the investigation on wheat rusts, specially for breeding of varieties for rust resistance. The occurrence of six races of Puccinia graminis tritici (Pers.) Eriks. & Henn., six of P. triticina Eriks. and eight of P. glumarum (Schm.) Eriks, & Henn. in India during 1932-38 had been reported by Mehta (1940). Gokhale and Patil (1952) reported race 122 of P. graminis tritici from Bombay State. Three more races of P.g. tritici, two of P. triticina, and two of P. glumarum have been found. Two of the new races of P.g. tritici which were provisionally labelled as "A" and "B" have now been given international numbers 194 and 117, respectively, and the new race "D" of P. triticina has been identified as a biotype of race 11. The new races of P. glumarum have been provisionally designated as races "G" and "H." The types of infection produced by these races on their respective differential hosts are given in Tables I, II and III. Race 117 is closely related to races 53 and 119 with minor differences only on Marquis.

TABLE I

Types of infection produced by new physiologic races of Puccinia graminis tritici on the differential hosts, (Stakman and Levine, 1922).

Race	Little Club	Marquis	Reliance	Kota	Arnautka .	Mindum	Splemar	Kubanka	Acme	Einkorn	Vernal	Khapli	Year of 1st observa- tion	
34 117 194	4 4	3,4 3,4 3,4	4 0; 0;	4 0; 3,4	3,4 3,4	4 3,4 3,4	4 4 3,4	4 4 0-1	4 4 0-2 or x	0-1 4 0-1	0-1 3-4 0-1	0-1 2 0-1	1939-40 1944-45 1944-45	

Table II

Types of infection produced by new physiologic races of Puccinia triticina on the differential hosts, (Johnston and Mains, 1932).

Race	Malakof	Carina	Brevit	Webster	Loros	Mediterra- nean	Hussar	Democrat	Thew	Year of 1st observa- tion	
11 26	0; 0;	2-3 4	3-4 4	0-1 0-1	2-3 3	0; 0-1	0; 3-4	0; 0-1	0-1	1944-45 1944-45	

TABLE III

Types of infection produced by new physiologic races of Puccinia glumarum on the differential hosts, (Straib, 1937).

Race	Michigan, Amber	Ble rouge d'Ecosse	Strubes Dickkopf	Webster	Holzapfel's Fruh	Vilmorin 23	Heines Kolben		Spaldings prolific	Rouge p.	Chinese 166	Triticum dicoccum tricoccum	Fong	Heils Franken	Petkuser rye	Year of 1st observation
G	0	0	0	0	0	0	4	0	0	0	0	4	4	0	0	1936-37
H	3	3	2-3	3	0	2-3	4	0	i	0	0	4	4	0	0	1939-40

Grateful thanks are due to late Dr. K.C. Mehta under whose guidence this work was done and to Dr. R.S. Vasudeva, Head of the Division of Mycology for very kindly guiding the preparation of this note. Thanks are also due to Prof. E.C. Stakman and Dr. C.O. Johnston for confirming the identification of the physiologic races of *Buccinia graminis tritici* and *Puccinia triticina*, respectively.

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A NEW LEAF BLIGHT OF CROSSANDRA INFUNDIBULIFORMIS NEES

M. K. PATEL, M. N. KAMAT AND C. B. PANDE (Accepted for publication July 2, 1953.)

Crossandra infundibuliformis, locally known as Aboli with its elegant flowers is widely cultivated in places close to big cities for market. The plant is a favourite in the coastal regions, where it occupies a prominent position in almost every kitchen garden. A severe leaf blight resulting in defoliation was noticed on this plant in Poona during the monsoon of 1950.

The chief characteristic symptoms on the leaf consist of brownish, depressed, necrotic areas surrounded by a reddish, slightly raised margin. The spots begin as small, round, brownish specks which become darker as they enlarge. The lesions, more prominent on the lower leaves, are largely confined to leaf margins (Plate 1, A). Severely affected leaves tend to roll up from the margin and get shrivelled in advanced stages. The disease ultimately kills the affected leaves and severe defoliation takes place, leaving a barren stem with a whorl of young leaves at the top. The disease does not attack other parts of the plant. The diseased leaf tissue yielded in culture a *Colletotrichum* sp.

PATHOGENICITY OF THE FUNGUS

A suspension of conidia was atomised on seedlings that had been kept 12 hours before in the moist chamber and subsequently for 72 hours. Yellow spots appeared 5 days later. At the end of 13 days, when infection had advanced spots turned dark and coalesced to form wider patches. The attack was mostly confined to margin of the leaf. The young succulent leaves were unaffected. The control plants did not show any symptoms of the disease.

MORPHOLOGY OF THE FUNGUS

The fungus produces profuse mycelial growth on Richards', oat meal and potato dextrose agar media. Mycelium is sub-aerial, thin, septate, hyaline containing droplets of oil globules, branching at an acute angle in most cases and measuring $4-6\mu$ in diameter.

Fructification. Abundant, minute clusters of acervuli of various shapes measuring $132\text{-}276\mu$ are formed on Richards' and potato dextrose agar media. The setae are irregularly arranged, dark greenish-brown throughout and are $97\text{-}147\times3\text{-}5\mu$ with 3-5 septa. The conidiophores are simple, short, hyaline, cylindrical or slightly swollen, usually septate at the base, rounded at the apex and measure $12\text{-}28\times3$ to 6μ . The conidia from cultures are hyaline, single but pale pink in mass, unicellular, varying in shape from cylindrical to elongated, oval, sometimes slightly curved

measuring $18-29\times1-5\mu$. (Fig. 1). The conidia from acervuli show slight differences from those obtained from the *Sporodochial* like mucilaginous bodies. The former contain rich protoplasmic material and fewer vacuoles than the latter. Terminal or intercalary thick walled, greenish-yellow chlamydospores are produced in old cultures.

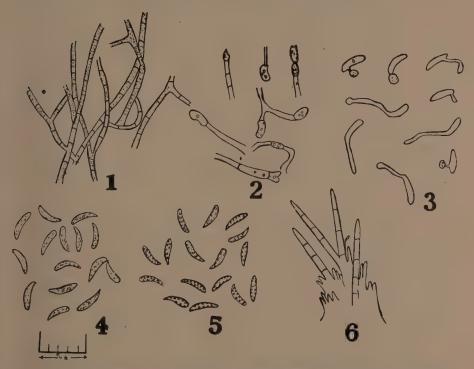


Fig. B. Morphology of the fungus:—(1) Branched septate mycelium. (2) Terminal and intercalary chlamydospores. (3) Germinating spores with appressoria. (4) Spores from acervuli. (5) Non-germinating spores from sporodochia. (6) Section of acervulus.

PHYSIOLOGY OF THE FUNGUS

Growth on various culture media. The fungus grew vigorously on oat meal, potato dextrose, lima bean, corn meal, artificial potato dextrose, and Richards' agars, but made scanty growth on host-decoction agar on which the sporulation and production of acervuli were profuse with the addition of dextrose.

Growth in relation to temperature. The linear growth of the fungus and rate of sporulation was studied in Richards' agar, incubated at various temperatures, and the results in Table I represent the average at the end of 7 days.

TABLE I

Growth and sporulation of Colletotrichum sp. at various temperatures

Temperature in °C	Colony diameter in mm.	Sporulation		
15	Trace	Nil		
20	48	Nil		
25	- 58	Scanty		
27-29 (roon	71	Profuse		
30	59	Profuse		
35	39	Moderate		
37	Trace	Nil		

The fungus grew vigorously at temperatures between 25° and 35°C., the optimum being 27°C., at which the sporulation was abundant. It failed to grow above 37° and below 15°C. Thus it is favoured by moderate temperatures and has a narrow range for growth and sporulation, (Plate 1, fig. B.)

Carbon sources: The fungus grew profusely on media containing glucose, sucrose and raffinose, moderately on lactose, glycogen, inulin, dextrose, maltose, rhamnose, amygdalin, mannitol, dulcitol, arabinose but made poor growth on galactose and salicin. Sporulation was best on media containing glucose, sucros, raffinose, dextrose, amygdalin and rhamnose; moderate in galactose, glycogen, inulin and maltose and scanty in salicin, arabinose, mannitol and dulcitol.

Nitrogen sources: The fungus grew best on modified Richards' agar containing sodium nitrate and peptone. These results are in general accord with those obtained by Mathur, Barnett and Lilly (1950) who worked with a gamma strain of Colletotrichum lindemuthianum. The media containing ammonium phosphate, asparagin, ammonium lactate and ammonium tartrate were suited for growth but not those containing 1-naphthylamine and sodium nitrite. A close correlation appears to exist between growth and sporulation on all the media except in ammonium nitrate and ammonium sulphate where growth was dense and profuse but the development of acervuli poor.

Hydrogen-ion concentration. The weight of the mycelial mat that had grown for 12 days in Richards' solution adjusted to different pH is recorded in the following table,

TABLE II

Growth of Colletotrichum sp. at different pH

pH	Dry weight of mycelium in mg.
1.90	. 0
3.10	275
4.20	700
5.30	720
5.90	1235
7.20	936
8.10	. 550
9.20	460

The fungus can grow in a wide range of H-ion concentration. The range of optimum reaction appears to lie between pH 6 and 7. In general, amount of mycelium formed is greater in acid than in alkaline media.

Enzyme production. For investigation of the enzymes produced by the fungus, various media as described by Crabill and Reed (1915) were taken advantage of. The results show that the mycelium secretes inulase, amidase, protease, emulsin, erepsin, trypsin, amylase, and cytase, which thus enable the fungus to utilise a wide range of food materials.

GERMINATION OF CONIDIA

During the course of these experiments, it was discovered that the spores produced in acervuli behave differently from those obtained from mucilaginous mass (sporodochia—like) in respect of germination since the spores from the former gave 76% germination with larger and vigorous germ tube as against a trace of germination with small and poor germ tubes from the latter.

It was next necessary to note if the conidia from sporodochial mass could be stimulated to germinate better with the addition of the host tissue. The result showed that the host tissue had certain stimulatory effect on germination which gave only a poor germination in distilled water and 33.5 per cent when host tissue was added.

The conidia from acervuli were used in all subsequent experiments since those from sporodochial mass gave poor germination. The effect of different temperatures on germination was tested with the following results:

TABLE III

Temperature in relation to germination

Temperature in °C	Per cent germination				
10. 15 20 25 26-28 30 35 37 41	Trace 16.9 63.85 76.38 80.20 67.51 58.75 17.45 0.00				

The minimum temperature for germination was 10°C. With further rise in temperatures, germination increased and was at its optimum at 26°-28°C. It then fell sharply and was completely suppressed at 41°C. The temperature range for germination of conidia is thus very wide. This is in accord with general abservations made in respect of development of disease in nature and behaviour of the fungus in culture.

Different media as given in the following table were tried to find a medium giving the highest per cent of germination.

Table IV

Effect of different media on germination

Medium	· Per cent germination
Dist. water	79.80
Dist. water+Host decoction	81.33
Dist. water + 1 per cent sucrose	86.44
Dist. water + green host tissue	90.38

The results show that there is very little stimulatory effect of host decoction on germination. The addition of I per cent sugar increased germination by 6 per cent while addition of green host tissue increased by 10 per cent. The differential effect produced by green host tissue and its boiled decoction is significant and shows that the stimulatory effect of the former is probably of biochemical nature.

HOST RANGE

The host range of the fungus has an intimate bearing on the nature of control and greatly helps in determining the taxonomic position, especi-

ally in highly specialised groups. Over a dozen hosts belonging to different families, both allied and remote were subjected to artificial inoculation with the fungus in the usual manner. The plants were Acanthus sp., Adhatoda vasica, Asteracantha longifolia, Barleria prionits, Capsicum annuum, Cicer arietinum, Curcuma longa, Gossypium arboreum, Hygrophalia serpyllum, Justicia gendarussa, Lycopersicum esculentum, Rhinacanthus communis, Ruellia ciliosa, Solanum melongena, Thunbergia grandiflora and Crossandra infundibuliformis. The fungus infected only the last showing that it is highly specialised.

TAXONOMY

The chief characteristics taken into consideration in defining a species in the group are morphological characters and the host range.

The species of *Colletotrichum* and *Gloeosporium* are very difficult to separate because many are morphologically variable and are rather weak parasites, a single species producing disease in a great variety of plants as shown by Shear and Wood (1907) and others. Ling and Lin (1944) state that "in comparison with a number of species of *Colletotrichum* such as *C. circinnans, C. indicum, C. truncatum, Glomerella glycinis, Colletotrichum capsici* differs from them in no essential way".

The dimensions of the acervuli fluctuate a great deal in the same isolate and consequently its size is not of much taxonomic value. Butler (1918) has recorded the size of acervuli of *Colletotrichum capsici* as $75\text{-}120\mu$. Ling and Lin (1944) state that the size of acervuli of *C. capsici* on one host varied from $97\text{-}288\mu$. The acervuli of the parasite under study measure $132\text{-}276\mu$. A structure which exhibits such wide variations can hardly be relied upon for specific differentiation,

The setae formed on the acervulus have been known to be definitely influenced by the environment and the substratum. Itaka (1937) and Ramakrishnan (1941) have indicated that the setae cannot be considered to be of any importance for the purpose of specific differentiations, as there is a wide variability found in this structure from isolate to isolate.

The shape and the size of the conidia only form important taxonomic characters. In the genus *Colletotrichum*, the shape of the spores is either oblong, spindle shaped, or falcate with tapering or blunt ends in different species but more or less constant in the same species. The size is, however, influenced by the substrate and varies within limits. Yet its significance in specific differentation cannot be ignored. Judged by these standards, it is seen that the isolate under study does not entirely agree with any of the *Colletotrichum* species described previously.

Some of the previous workers seem to have laid a greater emphasis on the pathogenicity in differentiating the species. Colletotrichum capsici was first recorded on Capsicum and Colletotrichum curcumae on Curcuma longa. Cross inoculation experiments by Sundararaman (1926) show that the fungi on Capsicum and Curcuma longa are the same.

Dastur (1934) erected provisionally a new species, Colletotrichum indicum causing seedling blight of cotton since he found that the isolate from cotton did not infect Capsicum nor did Colletotrichum capsici infect cotton seedling.

The foregoing considerations indicate that the shape and size of the spores and the pathogenicity of the organism are of important taxonomic value in this group of organisms and as the organism under study differs when compared with any of the species of Colletotrichum described and this parasite being the first record on Crossandra infundibuliformis, the establishment of a new species has full justification. It is, therefore, proposed to name it Colletotrichum crossandrae sp. nov. whose latin and English descriptions are as follows:

Mycelium $3.7-5.7~\mu$ diam., subaereum, hyalinum, guttulis, oleaceis praeditum. Acervuli glomerati, magnitudine et forma differentes. magnit. 132-276 μ . Setae irregulares, septatae, fusce viridi-brunneae, magnitud. $97-147x3-5~\mu$. Conidiophori simplices, breves, hyalini, cylindrici vel tenuiter tumidi, ut plurimum septati, rotundati ad apicem, magnitudinis. $12-28x3-6~\mu$. Conidia in acervulis singillatim producta, hyalina initio, demum pallide rosea in congerie, unicellularia, cylindrica vel elongata, ovalia, nonnumquam tenuiter curvata, magnit. $18-29x1-5~\mu$. Conidia in sporodochiis acervatim producta, pallide rosea, gelatinosa, tenuiter latiora, plus vacuolata atque rarissime germinantia. Inficit Crossandram infundibuliformem, cuius folia robigine corrumpit.

Typus lectus in urbe Poona, mense Novembri anni 1950, et positus in herbario "Plant Pathologist to Govern. Bombay, in Agricultural college, Poona; positus etiam in Indian Agric. Res. Instit., New Delhi, atque in Commonwealth Mycolog. Instit., Kew in Anglia.

Mycelium 3.7-5.7 μ in diameter, subaerial, hyaline, with oil drops. Acervuli in clusters, of different sizes and shapes, measuring 132-276 μ . Setae irregular, septate, dark greenish-brown, measuring 97—147x3—5 μ . Conidiophores simple, short, hyaline, cylindrical or slightly swollen, many septate, rounded at apices, measuring 12-28x3—6 μ . Conidia in acervuli produced singly, hyaline, pale rose coloured in masses, unicellular, cylindrical or elongate, oval, sometimes slightly curved, measuring $18-29 \times 1$ —5 μ . Conidia from sporodochia-like acervuli are pale rose, gelatinous, sparingly produced, with many vacuoles and germinate sparingly. Pathogenic to Crossandra infundibuliformis causing dark necrotic spots on leaves.

Type material collected at Poona in November, 1950 and deposited in the herbarium of the Plant Pathologist to Government, Bombay, Agricultural College, Poona; also deposited in the herbaria of Indian Agricultural Research Institute, New Delhi and Commonwealth Mycological Institute, Kew, England.

SUMMARY

A detailed study in morphology and physiology of Colletotrichum sp. causing a leaf blight of Crossandra infundibuliformis has been made.

High humidity and moderate temperature (25°-30°C.) favoured the development of the disease. The fungus has a wide range of temperature for its growth and sporulation (15°-27°C).

The fungus produces two types of conidia, those in acervuli germinate readily and those in sporodochia sparingly. The minimum,

optimum and maximum temperatures for germination are 15°, 27° and 37°C. respectively.

The fungus is highly specialised and being new to science is named Colletotrichum crossandrae sp. nov.

ACKNOWLEDGEMENT

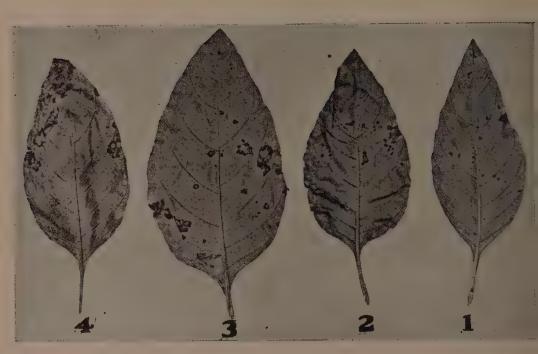
The writers are grateful to Dr. B. B. Mundkur for helpful suggestions and to Rev. Fr. H. Santapau for latin diagnosis.

Plant Pathological Laboratory, College of Agriculture, Poona 5.

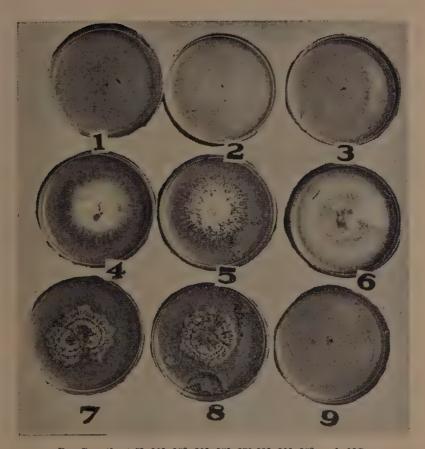
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A. Diseased leavas showing different stages of symptoms.



B. Growth at 5°, 10°, 15°, 20°, 25°, 27°-29°, 30°, 35°, and 41°C.

A NEW LEAF SPOT DISEASE OF BAJRA (PENNISETUM TYPHOIDES STAPF AND HUBBARD) CAUSED BY A SPECIES OF PIRICULARIA

P. R. Mehta, Babu Singh & S. C. Mathur (Accepted for publication July 25, 1953)

A new leaf-spot disease of bajra (Pennisetum typhoides) was observed during August-September 1952 at the Government Research Farm, Kanpur. Preliminary microscopic examination revealed the presence of a species of Piricularia. The disease was noticed on inbred lines of the crop in varying degrees of severity and some lines were severely affected. The senior author had noticed this disease as early as 1942 on leaves of plants affected by "downy mildew" but as the disease was never seen on healthy plants, no attempt was made to further investigate the disease. On the inbred lines, however, the lesions were big and the damage fairly extensive. In many States, experiments are now in progress to evolve inbred lines of this crop and even to release hybrid-seeds. It is, therefore, considered desirable to record this new disease and draw the attention of breeders and Plant Pathologists to the inherent danger of a disease of bajra which has so far been inconspicuous but may assume serious proportions if careful selection is not made of the breeding material.

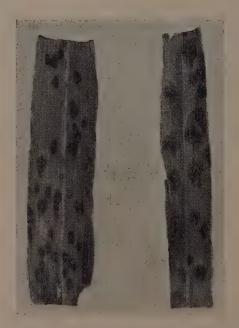


Fig. 1-Spots on leaves of bajra caused by Piricularia sp.

The disease first appears on the lower leaves of the plants as small light brown spots which enlarge into prominent dark brown circular spots usually 0.2 cm to 0.6 cm in diameter but some spots extend upto 1.0 cm. In some cases, the spots coalesce forming elongated and somewhat irregular lesions. Concentric rings of light brown to dark brown colour usually 2 to 7 in number are formed on the spots giving the appearance of zones. The formation of conidiophores and thereby of conidia takes place on the concentric rings. The disease is distinguished from other spot diseases known on bajra by the presence of concentric rings on the circular spots. (Fig 1).

The spores of the fungus are top-shaped, hyaline, mostly three-celled, straight or slightly bent measuring 17.6 μ to 30.8 μ in length and 5.9 to 8.8 μ in width with a small appendage at the broader base cell

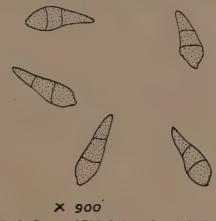


Fig. 2-Spores of Piricularia sp. from bajra leaf.

(Fig 2). The spores are produced on both the surfaces of the spots, and are borne in a scorpioid manner on conidiophores which are faintly brown with prominent septa. The conidiophores emerge through the stomata on the surface of the affected parts of leaves.

Ramakrishnan (1948) has recorded genus Piricularia on rice, Eleusine coracana Gaertn. Setaria italica Beauv, and Digitaria marginata Link and has given his findings on morphology, physiology and parasitism of the isolates from the above mentioned hosts. He concluded that the morphological characters did not afford much basis for their classification. He has also disagreed with the findings of Nishikado (1927) in the separation of the species, Piricularia setariae Nishikado from P. oryzae on the basis of morphological characters. On the basis of cross inoculation studies, Ramakrishnan (1948) concluded that the isolate from rice is distinct from all other isolates in the fact that it infects only rice. The isolates from other hosts infected their own hosts as well as one or two others. The isolates from Digitaria marginata Link, however, infected rice and few other hosts.

The isolates from bajra and paddy were used for pathogenicity and cross inoculation experiments by the authors. About twenty seedlings

of each host were inoculated and the experiment was repeated thrice in the season. The results are given in table $\Gamma:$ —

TABLE I

Showing the Results of Pathogenicity and Cross Inoculation Experiments with Piricularia Isolates from Bajra and Paddy

	Hosts					
Piricularia · isolated from	(P. typhoides				
	T100	T136	T21	1. typhoides		
1. Pennicetum typhoides	Brownings.			- -		
2. Oryza sativa	+	+	+	_		

The disease appeared within 4 to 6 days in more than eighty per cent of inoculated bajra plants. It may be concluded from the results that the isolates from bajra do not infect paddy and vice versa. This along with a distinct difference in the width of the conidia of the two isolates leads to a strong suspicion that the fungus on bajra may be a

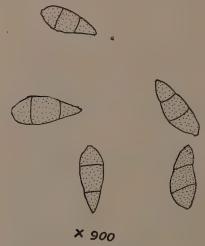


Fig. 3—Spores of Piricularia Oryzæ from paddy leaf.

new species. Further-more apical cell of the bajra isolate is distinctly longer and tapering than that from the isolate on rice (Fig 3). It is proposed to carry out a detailed study of the fungus by using cross inoculation tests on other hosts known to be affected with *Piricularia* viz. *Eleusine coracana* maize, *Setaria* sp., *Digitaria* sp., *Panicum* sp., etc.

It may also be stated that the "blast disease" of paddy has not been observed at the Experimental Station Farm, Kanpur nor has a search been made to find out if *Piricularia* occurs on other cultivated and wild host at the Government Research Farm with a view to find out the hosts range of *Piricularia* at Kanpur.

The writers are grateful to Shri T.S. Ramakrishnan, Mycologist to Government, Madras for the supply of specimens of blast affected paddy leaves and cultures of *Piricularia* on *Oryza sativa* and *Eleusine coracana* and for his helpful suggestions. They are also thankful to Dr. D.P. Singh, Assistant Economic Botanist (Pulses, Millets and Oilseeds) of the U.P. Government for drawing their attention to this disease.

Laboratory of the Plant Pathologist to Government, U.P., KANPUR.

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INDIAN WILD LINSEED (LINUM MYSORENS HEYNE), A POSSIBLE COLLATERAL HOST OF RUST OF THE CULTIVATED LINSEED (LINUM USITATISSIMUM L.) IN THE HILLS.

V. C. LELE.

(Accepted for publication July 26, 1953)

A few plants of yellow-flowered linseed *Linum mysorens* Heyne were found growing wild on grassy slopes about two miles South of Simla at an altitude of 6,500 ft. in early September 1950. The seed was collected for study during October-November. Duthie (1905) has recorded the distribution of the species at Mt. Abu in Rajputana, Western Himalaya (3-5,000 ft.) and south-ward to the Deccan and Ceylon. Collect (1921) recorded the species to occur within fifty miles in the neighbourhood of Simla as also Western Himalaya 3,000 to 5,000 ft. and hilly districts throughout India.

Prasada (1948) observes "It may, however, be stated that infected wild species of Linum had not been recorded in India so far."

Wild Linums as collateral hosts of flax rust and their role in the perrenation of rust all the year round has been studied in many countries. Hart (1925, 26) found that the uredospores from L. usitatissimum did not infect L.lervisii and vice versa, but those of the former heavily infected L. rigidum. Waterhouse and Watson (1944) have recorded the wide spread natural infection of L. marginale, which is a perenial wild plant in Australia. Cass Smith and Harvey (1946) observe that "the wide distribution and perenial habit of L. marginale caused it to be regarded as an important potential source of M. lini and thus a danger to commercial crops in Australia." Straib (1939) found that two of the races of M. lini tested (Swedish and German) attacked, in addition to L. usitatissimum, its var. crepitans, 7 species of Linum, while 25 other species remained free from infection. Ware and Glasscock (1943) on the other hand, found L. catharticum, a common field weed in England, not susceptible to the physiologic race of M. lini attacking cultivated flax.

Though search for rust on wild species around the place of its original collection proved unsuccessful, a thorough search in the lower range (3,000-5,000 ft.) of the Simla hills where linseed (*Linum usitatissimum*) is regularly cultivated (collett, 1921) may lead to naturally infected plants of the wild sps. That no natural rust infection was observed may be accounted to the fact that linseed is not grown in the adjacent neighbourhood of Simla. The wild species was found flowering near simla (6,500 ft.) in early September and there was evidence that it has been flowering during the latter part of August. In lower ranges the flowering period may appear to be still earlier, as has been recorded by earlier workers.

Seed collected in October-November, were sown in pots in glasshouse in February. But probably on account of severe cold, no germination was obtained. Subsequent sowings in July resulted in 80 per cent germination

and healthy growth of seedlings. About 20 seedlings were inoculated with uredo material of all the four Indian Physiological races in mixture, and moderate to heavy infection of susceptible type was obtained on cotyledonery and other leaves. The temperature varied from 58 to 70°F, and rust appeared within 10-12 days. Rust from infected wild species was successfully transferred to cultivated Punjab local linseed. No necrosis or chlorosis was found on plants of wild species which had not been infected.

Linseed is sown in October-November and harvested in March-April in the plains of India. It has been shown by Prasada (1948) that rust can over-summer as well as over-winter in the hills and that given a congenial host from the time of harvest to the next crop, the disease could be carried over in the uredial state. According to him the crops in the hills get infected first and then fresh uredo-spores are blown by wind to the plains causing fresh outbreaks.

Besides self-sown or volunteer plants of cultivated linseed, wild linseed *L. mysorens* Heyne appears to be a congenial host for perrenation of rust in the hills all the year round, as the period of its growth inter venes the harvest of previous crop and sowing of the next, *i.e.*, May-October.

Rust Research Laboratory. Simla, Indian agricultural Research, Institute, New Delhi.

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BACTERIAL LEAF SPOT OF AMARANTHUS VIRIDIS L.

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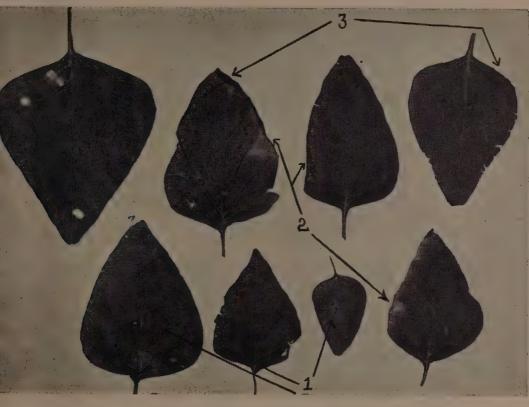
Introduction

In February, 1952, a bacterial leaf spot on Amaranthus viridis L., a common weed growing on the roadside and waste lands was observed on the Agricultural College Farm. On further observation, the disease was found quite prevalent in Bombay State. Smith (1914) was first to report a bacterial disease on Amaranthus sp. in a neglected garden at Mumford, New York, in 1897. In his note, he stated "the disease has not come again under my observation; and no plate cultures were made and that the organism may be known as Bacterium amaranthi." Burns, who according to Smith (1914) was second to find the disease on two cultivated Amaranthus spp. at Ann Arbor, Michigan, in 1901, plated out a yellow organism but having read Smith's note dropped further work on it. In view of the fact that Burns had abandoned the work and no isolations were made by Smith, since he merely streaked the organisms obtained from the interior of the stems on Loeffler's blood serum without proving the parasitism of the organism, it was thought worthwhile to study the organism obtained from the leaves of A. viridis at Poona and to see whether the same organism caused the disease here. The results of the morphological, cultural and biochemical characters of the organism reported briefly by Patel, Wankar and Kulkarni (1952) are recorded in detail in the following pages.

DESCRIPTION OF THE DISEASE

In nature, the disease was noticed only on leaves on which the pathogen produces a few water-soaked spots on the lower side. After about 5-6 days, the spots become visible on the upper surface of the leaf as pale brown, round areas surrounded by a yellow zone or halo. These water-soaked spots when viewed against light look translucent and measure 0.5 mm. On the advancement of the disease, these spots become deep brown and depressed on the lower surface; on the upper surface, the margin of the spots is raised, while in the centre, in many cases the spots become parched as if lacerated by insects. Often the spots coalesce and form irregular lesions measuring 1.5-2.0 mm. (Plate 1). The infection is invariably found along the edge of the leaf. Bacterial ooze is sometimes found in the centre of the spots.

The stem and petiole get infected under favourable conditions of humidity and temperature in artificial inoculations when the watersoaked areas appear around the injured portions. In advanced stage of the disease, sides of the stem get cracked becoming jet black while the central portion remains grey. In the case of petiole, the colour of the cracked portion remains brownish,



Symptoms on Amaranthus (leaves) Bacterial leaf Spot of Amaranthus viridis L.

ISOLATION OF THE CAUSAL ORGANISM

The pathogen was isolated by the usual method on potato dextrose agar plates on which after 2 to 3 days, pale yellow, glistening colonies appeared. The pathogenicity was proved by inoculation on healthy plants of A. viridis and the organism resembling the original culture reisolated from the leaf. The reisolated culture was used in these studies.

INOCULATION EXPERIMENT

On inoculation, small, water-soaked spots around the punctured and non-punctured areas on the leaves appear after 4-5 days. These spots in the course of other 2-3 days become pale brown and finally deep brown in 10-15 days. The margin of the leaves also gets infected invariably and become deep brown. In very advanced cases, the leaf surface becomes parched and papery in consistency. Control plants remained healthy.

Inoculation on tender stem was done after making a scratch by a sterilized needle and spraying with the pathogen. Small, vertical, watersoaked streaks began to appear round the injured portion which turn greyish when cracking is noticed. The sides of the cracks become jet black whereas the central portion remains grey. On inoculation of petiole, the water-soaked areas with cracking appeared where a scratch was made but the colour remained brownish.

MORPHOLOGY AND STAINING REACTIONS

The pathogen is a short rod with rounded ends, mostly single, rarely in chains of two, with no involution forms. In cultures on potato dextrose agar varying in age from 1-3 weeks, the average dimensions are $1.35-2.1 \times 0.6-1.3 \,\mu$. It is motile by a single polar flagellum, Gramnegative, non-acid fast, capsulated and non-spore former. It stains readily with common dyes.

CULTURAL AND PHYSIOLOGICAL CHARACTERS

The cultural characters of the organism were studied at 31°C. on various media using ingredients of the highest purity. On potato dextrose agar plates, the colonies are smooth, round, pulvinate, glistening, but vrous with no marked odour and the colour Empire vellow (R). colony measured 1.5 cms. at the end of 7 days with no internal striations. On potato dextrose agar slants, growth is copious, raised, smooth, shining, filiform; butyrous in consistency, spreading at the base of the slant with no marked odour and the colour Empire yellow (R). On potato cylinders, growth is copious, raised, shining, and the colour primuline yellow (R), flowing and covering the entire surface in 5 days with no colour change, while on nutrient agar slants and plates, the growth is poor, flat, thin, glistening, primuline vellow (R) with no distinctive odour and the colour unchanged. In nutrient broth, there is slow growth in 4 days with moderate clouding and no pellicle or sediment. It fails to grow in Cohn's and Uschinsky's solutions. Moderate reduction of litmus was observed after 10 days while the medium was slowly cleared, the colour turning to Roseline purple (R). Liquefaction of Loeffler's solidified blood serum started slowly after 3 days and was almost complete in 15 days. There was very slight growth in Fermi's solution. It liquefies gelatin completely and digests starch. Although unable to utilise cellulose, it made good growth on casein and egg albumen. Hydrogen sulphide was produced. Nitrate was not reduced to nitrite but ammonia was produced from peptone. Indole was not produced. It made good growth in 1 and 2 per cent, slight growth in 3 per cent but failed to grow in 4 per cent sodium chloride solution. It grows well on Endo's agar, and Simmon's citrate agar but poor growth in Koser's uric acid medium. Best growth between 27° and 31° C., while no growth occurred at 40° C. and 5° C. Good growth with acid production in arabinose, galactose, dextrose, lactose, levulose, maltose, sucrose, glycerol, mannitol and xylose; slight acid with fair growth in raffinose; no growth in salicin and oxalic acid; and good growth with alkaline reaction in acetic and citric acids. The thermal death point near about 51° C. Aerobic. It lives for 21 months at 13° C. on sterilised glass beads as against 8 days in unsterilised soil and for 51 months in sterilised soil at 26° to 28° C.

HOST RANGE

Amaranthaceae is quite a large family and hence to determine the host range, plants of Amaranthus spp. and related genera grown in sterilised soil in 4" pots were inoculated when 3 weeks old by puncturing both the stems and the leaves with sterile needle. The pathogen infects leaves and stems of Amaranthus viridis, A. blitum, A. caudatus, A. gangeticus, A. mangostanus, A. polygamous, A. paniculatus, A. splendens, but failed to infect A. spinosus and Amaranthus spp. (moltenfire and rainbow mixture), Achyranthes aspera, Celocia cristata (nana mixed), C. cristata (nana class), C. pyramidalis and C. conzoides.

TAXONOMY AND NOMENCLATURE

The study on morphology, cultural and bio-chemical reactions of the organism shows that it differs from *Bacterium amaranthi* Smith (1914). In table I, a statement giving some similarities and other distinguishing cultural and physiological characters of the two organisms is presented.

Table I

Comparison of Bacterium amaranthi with Xanthomones amaranthicola

	B. amaranthi	X. amaranthicola
Morphology	Short rods with rounded ends, no long chains.	Short rods with rounded ends, mostly
Motility	Actively motile.	single, Motile by polar flagellum,
Spore	Filaments or endo-spores	No endo-spores.
Physiological characters (1) Potato cylinders	The growth was homogenous, wet looking (shining), thin, translucent. The colour was first pale yellow and distinctly ochraceous. The substratum was stained decidely gray. (not	Growth copious, raised, shining, primuline yellow colour (R). The colour of the potato cylinder not darkening.
(2) Starch digestion	brown), drab gray. On mashing old potato cultures in iodine and potassium iodide water there was copious brown purple reaction, showing thereby that the starch had been acted	Addition of iodine solution in alchohol indicated that the organism had strong digesting power for
(3) Loeffler's blood serum	upon only a little. At first a dirty white or pale yellowish white, wet, (shining) growth. This subsequently became yellow with a rather copious yellow precipitate at the bottom of the tube. No liquefaction was observed.	starch. The organism grew well and there was strong evidence of liquefaction after 15 days.

It seems quite evident that the organism under study differs from B. amaranthi in (1) absence of spore, (2) production of yellow pigment on solid media, (3) liquefaction of Loeffler's blood serum and (4) strong power of digesting starch. By far, the major difference lies in symptoms produced by the two organisms. Smith (1914), while reporting on B. amaranthi, stated that the plants seemed to have dried up when halfgrown, with no surface indication as to the cause of the disease. The stems were browned internally and the cavities in the parenchyma were full of bacteria in the region of vascular ring, resulting in drying up and wilting due to choking of parenchymatous tissues. In the case of Xanthomonas amaranthicola, there was clear indication of the disease being of bacterial origin and the stems though infected with the organism showed no drying up or wilting.

DISCUSSION AND SUMMARY

The organism causing leaf-spot of Amaranthus viridis differs from the one described by Smith in cultural and bio-chemical characters besides being not vascular. The disease was observed by Smith once only and there is no mention of parasitism being proved. Smith has further stated that Amaranthus plants were weeds standing on what had been a cabbage seed bed. In order to make sure that A. viridis was not an additional host of X.campestris, leaves and stems of A. viridis were inoculated with X. campestris. Also X. amaranthicola was cross inoculated on cabbage seedlings. In both cases, the organisms were found restricted to their own hosts.

Smith noted wilting of plants due to *B. amaranthi* which in the present case was never observed. To a very few instances wherein a bacterial pathogen infects more than one species as in *X. campestris*, *X. citri*, this adds another instance where the pathogen infects leaves and stems of ornamental and flowering plants belonging to seven other species of *Amaranthus*.

From the morphological, cultural and bio-chemical study, it is inferred that the pathogen belongs to the genus *Xanthomonas* and hence named *Xanthomonas amaranthicola* sp. nov.

TECHNICAL DESCRIPTION OF

Xanthomonas amaranthicola sp. nov.

Short rods; single or rarely in chains of two; single polar flagellum; $1.3 \times 0.6 \mu$; Gram negative; capsulated; no spores; on potato dextrose agar plates, colonies are circular with entire margin, smooth, shining, pulvinate, striations absent, measuring 1.5 cms. at the end of 7 days, colour Empire yellow (R); gelatin liquefied, starch hydrolysed; casein and egg albumen utilised; milk peptonised; litmus reduced; hydrogen sulphide and ammonia produced; nitrate not reduced; acid but no gas in dextrose, maltose, sucrose and lactose; no growth in salicin; optimum temperature for growth 27° to 31° C.; thermal death point near about 51° C.; pathogenic to Amaranthus viridis, A. paniculatus, A. blitum, A. caudatus, A. gangeticus, A. mangostanus, A. polygamous and A. spledens producing leaf-spot and attacking stem. Found at Poona and Bombay city.

The writers express their deep gratitude to Dr. S. P. Capoor for help in photographic work.

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INVESTIGATIONS ON THE SURVIVAL OF COLLETOTRICHUM FALCATUM WENT IN SOIL

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I. Introductory

The survival of Colletotrichum falcatum Went, the causal organism of Red Rot disease of sugarcane, in the soil under field conditions and the role that soil infection may play in the life history of the organism has not been extensively investigated. Butler (1906) stated that "the spores from a vigorous culture buried in the soil at a depth of 3 inches, were found to be all dead or germinated after 8 weeks, and the mycelium all killed. On the other hand, cultures placed freely exposed on the surface of the ground, survived in dry weather and germinated freely after the same period." Later, he (1907) observed that the fungus can live in the soil or on decaying leaves in the absence of the cane, but it could not survive for more than three to four months. Butler and Hafiz Khan (1913) found that the fungus died out rapidly in the moist soil but cultures exposed to the air and kept moderately dry retained their viability upto five months. Abbott (1938), working on the disease in Louisiana, observed that while it seemed possible that the fungus may be able to survive for a considerable time on buried trash in the soil and could presumably infect seed cane, this source of inoculum was relatively unimportant at least in the United States. He (1926), however, failed to isolate C. falcatum directly from the soil by ordinary methods of plating soil suspension. Chona and Padwick (1942) showed that the infection of the planted setts and shoots arising from them can take place through the infected soil. Chona (1950) further observed that the survival of the Red Rot organism in the soil in the form of cane debris or as pure culture was limited to 5 months only. In order to know the cause of the limited survival of *C. falcatum* in the soil, it was proposed to investigate the effect of soil micro-organisms on C. falcatum in soil and the suitability of the soil as a medium for its growth. The results of the investigation are reported in this paper.

II. EXPERIMENTAL

Effect of soil micro-organisms on the survival of C. falcatum in the soil:— To test the effect of soil micro-organisms on the survival of C. falcatum in soil; the organism was grown in sterilised and unsterilised soil cultures and these soil cultures were, from time to time, inoculated into healthy canes of a susceptible variety. Attempts were later made to re-isolate the fungus from these inoculated canes and to establish thereby the survival of the fungus.

The soil cultures were prepared in the following manner: The soil was obtained from a fallow plot in the field which had not been planted with sugarcane for the last three years. The soil was sieved through a fine sieve, weighed and distributed in test tubes in lots of 7 grams. Two c.c.

of water was added to each tube to provide the necessary moisture. A second lot of test tubes was filled with 7 grams of a mixture of soil and well rotted compost (Farm Yard Manure) in each and 2 c.c. of water added. The mixture of soil and compost was prepared by mixing 4 parts of soil with one part of compost. Twenty tubes were prepared of each set. Half of the tubes of each set were sterilised twice in the autoclave for one hour at 20 lbs pressure and the other half were left unsterilised. Both the sterilised as well as the unsterilised tubes containing soil or soil-compost mixture were inoculated with isolate 78, a viroulent, light coloured and highly sporulating type of C. falcatum.

Growth in soil cultures:—Weekly observations were taken regarding the growth of the fungus in the soil cultures. After 4 weeks growth, the cultures were examined microscopically. It was observed that C. falcatum is capable of growing in both the sterilised soil and soil-compost mixture and that the growth of the fungus is more profuse and fast in the latter than in the former. Nothing could, however, be said with certainty regarding the growth of the fungus in the unsterilised soil or soil-compost mixture, as no C. falcatum hyphae or spores could be detected amidst the overwhelming growth of the other soil saprophytes. Inoculation tests were, therefore, carried out with these cultures into healthy canes of a susceptible variety, namely, Co. 299.

Inoculation tests:—To test the survival of C. falcatum in the soil, inoculations were made in healthy canes of Co. 299, a cane variety highly susceptible to Red Rot, with the soil cultures at desired intervals. With the help of a sterilised cork borer, a small hole was punched in a middle internode of the cane after surface sterilisation and a small quantity of the soil-culture inoculum was carefully introduced into the cavity with a sterilised inoculating needle. The plug was then replaced in position and a strip of paper was wrapped round the point of inoculation and tied tightly with Sutli to prevent extranuous contamination. The inoculated canes were then stored in sterilised moist gunny bags and kept at room temperature (27-32°C).

The inoculations were made with one month, 3 months and 4 months old cultures of C, falcatum growing on sterilised and unsterilised soil as well as soil-compost mixture. The nature and the extent of reddening produced in the inoculated canes was recorded $2\frac{1}{2}$ to 3 months after the inoculation by cutting open the canes longitudinally. Attempts were made to re-isolate the fungus by placing small pieces of the infected portion of the inoculated canes in moist chambers. The results of the inoculation tests are given in Table I.

It is clear from Table I that *C. falcatum* cultures growing on unsterilised soil and soil-compost mixture are capable of causing infection of the canes upto three months only, while in the cultures growing on the sterilised soil or soil-compost mixture the fungus is present in a viable form even after four months. Further inoculation tests could not be carried out due to the cane season being over and there being no mature canes available for inoculation. The survival of the fungus in these cultures beyond four months was tested by direct transfer and viability tests of the spores.

TABLE I

Results of the inoculations of healthy canes of Co. 299 with soil-cultures of C. falcatum

	Inoculum	Age of culture (months)	No. of canes inoculated	No. showing Red Rot infn.
C. fall	catum on sterilised soil	1	5	5
,, ;	, on sterilised soil-compost			
	mixture	1 .	5	5
22 1	, on unsterilised soil	1	5	. 5
99 3	, on unsterilised soil-compost			
	mixture	1	5	. 8
27	on sterilised soil	3	10	. 8
29 .	, on sterilised soil-compost			
	mixture	3	10	9
22 1	, on unsterilised soil	3	10	5
,, ;	on unsterilised soil-compost			
	mixture	3	10	6
22 2	, on sterilised soil	4	6	5
22 2	, on sterilised soil-compost			
	mixture	4	61.	4
,, ,	, on unsterilised soil	4	6	0
	on unsterilised soil-compost			
	mixture	4	6	0 _

Direct transfer:—Besides the inoculation of canes, direct transfers were made every month from the cultures growing on soil and soil-compost mixture maintained at 27—32°C on Oat meal agar slants and incubated at 28—39°C to test the viability of the fungus. The cultures on the sterilised soil or soil-compost mixture gave C. falcatum growth upto fifth month, but after that period the sterilised soil failed to give any successful subculture of C. falcatum while the sterilised soil-compost mixture continued to yield C. falcatum up to the sixth month. Transfers made after six and half months interval failed to give any C. falcatum growth.

Viability of the spores:—The cultures on the sterilised soil-compost mixture showed abundant formation of C. falcatum spores on the surface and crevices of the substratum in the form of pink spore masses. Viability tests were carried out with these spores every month at a temperature of 28—30°C. It was found that the spores were viable in the sterilised soil cultures up to five months and in the sterilised soil-compost mixture upto six months, but failed to show any germination after that period.

These preliminary experiments clearly indicate that the Red Rot organism in the form of pure culture can survive in unsterilised soil or soil-compost mixture upto three months, while in sterilised soil it can remain viable for five months and in sterilised soil-compost mixture upto six months.

Suitability of soil as a medium for growth:—To test the suitability of soil as a medium for the growth of C. falcatum, the fungus (isolate 78) was grown on soil extract agar, soil-compost extract agar, plain agar and Oat meal agar media (Oats, 40 gms.; agar agar, 20 gms.; distilled water, 1,000 c. c.) and the growth of the fungus on these media was compared. The soil extract agar and soil-compost extract agar media were prepared in the following manner: 200 grams of soil-compost mixture (4:1) and 200 grams of soil as such were taken in separate flasks and 200 c. c. of water added to each. The flasks were then steamed in Koch's steriliser for one hour and kept overnight to bring all the water-soluble nutrients into the solution. The solutions were then decanted and filtered through filter paper and and the soil and soil-compost extracts were thus obtained. To these extracts, 2 percent agar was added and sterilised at 15 fbs pressure for 20 minutes to make soil-extract agar and soil-compost extract agar. Ten tubes of each were inoculated with C. falcatum, insolate 78, and incubated at 28—30°C. Weekly observations were taken for the comparison of the gorwth of the fungus on these media. The results obtained are presented in Table II.

TABLE II

Comparative growth of C. falcatum on plain agar, soil extract agar, soil-compost extract agar and Oat meal agar

to the state of th					
Age of Cultures (days)	Plain agar	Soil-extract agar	Soil-compost extract agar	Oat meal agar	
7	Very scanty growth; hyphal threads spreading on the surface	Scanty growth of mycelial threads on the surface	Fluffy greyish cobweb like growth about ½ cm. in diameter	Copious grow- th of fluffy mycelium filling the entire surface. Pink masses of spores begining to appear.	
14	Growth dying and subsiding	Scanty grow- th spreading over the entire surface	Fluffy growth increasing in all directions, surrounding the point of inoculation	Copious growth with abundant pink masses of spores	
21	Growth hardly visible	Growth dying and subsiding. Formation of a black incrustation at the base inside the medium	Fluffy growth of mycelium constant	do	
28	No growth visible	The black incrustation is increasing. No growth found on the surface	Growth starts drying up. Black incrustation started at the base	do	

In plain agar cultures, the fungus hyphae started dying in the second week of growth due, probably, to lack of food material. The soil-extract agar gave only slightly better growth than that obtained on plain agar. Though the growth started dying off about the third week, a black incrustation of chlamydospores appeared at the base, at the point where the medium touched the inner wall of the tube.

Chlamydospore formation was observed in the soil-compost extract agar also but slightly later than that in soil-extract agar as the former medium contains comparatively more of food material due to the presence of compost extract. The amount of growth on this medium is also greater than that on the soil-extract agar, but much poorer as compared with that on Oat meal agar medium showing thereby that soil is a poor medium for the growth of *C. falcatum*.

III. Discussion

The growth of C. falcatum in sterilised soil and soil-compost mixture has clearly shown the development of C.falcatum hyphæ and the spores. The development of pink spore masses on the surface of these cultures indicates that the fungus is capable of growing in the soil. On the other hand, the complete absence of the pink spore masses of C. falcatum in the unsterilised soil and soil-compost mixture and the fact that no hyphæ or spores of C. falcatum could be traced on microscopic examination in these cultures after one month's growth would give an indication of the anhilation of the fungus, C. falcatum, in these cultures. It is possible, however, that the fungus may be present but may not be in an active form due to the competition offered by the other soil micro-organisms; or it may have been missed in the overwhelming growth of the other actively growing soil fungi. From the inoculation experiments it is evident that the fungus is capable of survival for three months in the unsterilised soil and soil-compost mixture. nature we always have conditions of growth as that of mixed cultures of several micro-organisms and it is therefore presumed that the fungus can live for about the same period i. e. three to four months, in the soil under natural conditions. This is in accordance with the findings of Butler (1907) and Chona (1950). The fungus was, however, found to remain viable in the cultures on sterilised soil and soil-compost mixtures even up to the fifth and sixth month respectively. It may be concluded, therefore, that the greatly limited survival of the Red Rot organism in the unsterilised soil is due to the presence and interaction of other soil micro-organisms.

Satisfactory development of *C. falcatum* hyphæ and abundant production of pink spore masses in the sterilised soil-compost mixture, as compared with the sterilised soil alone, suggests that the growth is favoured by the addition of compost to the soil. The compost seems to serve as additional food material resulting in profuse growth of the fungus, showing clearly that soil by itself is comparatively a poorer medium and not very suitable for the growth of *C. falcatum*.

Thus the limited survival of *C. falcatum* in soil may be attributed partly to the presence of the other soil micro-organisms which offer a keen competition and partly to the fact that the soil, being a poorer medium, is not particularly suitable for the growth of *C. falcatum*.

Grateful thanks are due to Dr. R. S. Vasudeva, Head of the Division, for his kind interest and helpful criticism.

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THE PERITHECIAL STAGE OF COLLETOTRICHUM FALCATUM WENT IN INDIA

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Carvajal and Edgerton (1944) proved that the perfect stage of Colletotrichum falcatum Went, the causal organisum of Red Rot of sugarcane, is Physalospora tucumanensis, originally described by Spegazzini (1896). They found the perithecia of the fungus in Louisiana on Saccharum officinarum L., S. robustum, S. barberi Jesweit, S. sinense Roxb., S. spontaneum L. and Leptochloa filiformis (Lam.) Beauv. Outside the United States the perithecial stage has been recorded only from Formosa (Ling and Ma, 1951).

In our cultural studies on the variations of some Indian isolates of C. falcatum, it was noticed that certain light type, profusely-sporulating strains of the fungus produced sterile perithecia-like structures when grown on sterilised cane juice diluted with two parts of water for about 20 days at 20-25°C. This suggestive evidence was followed up by inoculation of the fungus on autoclaved pieces of leaf and leaf sheath of sugarcane. Perithecia, agreeing essentially with those described by Carvajal and Edgerton (loc. cit.), were produced when such inoculated material was incubated for about 20 days at 20-25°C. under conditions of high humidity. Similarly inoculated unautoclaved, surface-sterilised pieces of leaf and leaf sheath failed to develop any perithecia under identical conditions of incubation.

Mature perithecia appear as black dots scattered throughout on the surface of the substratum. Those formed on the leaf lamina are irregular in shape and are mostly submerged in the host tissue with the ostiole protruding outside, whereas, on the leaf sheath they are often globose and superficial with the bottom embedded in the host tissue. The average diameter of the perithecium is 250μ with a range of $150-300\mu$ (Fig. 1). The asci are numerous, hyaline and clavate, ranging from $49.0-66.5\times7.0-10.5\mu$ with an average of $56.2\times9.0\mu$ (Fig. 2). Paraphyses are numerous, hyaline, extremely delicate and disintegrate as oil drops on maturity. Ostiolar periphyses are also present. The ascospores are eight in number and are arranged biserially within the ascus. They are single celled, hyaline, elliptical and measure $17.5-21\times5.3-7.0\mu$ with an average size of $19.6\times6.3\mu$.

Though the natural occurrence of the perfect stage of the fungus has not been detected so far in India, the readiness with which the perithecia are formed under simple laboratory conditions, suggests the possible existence of the ascigerous stage under field conditions and the occurrence of new virulent strains of the fungus in the country. If the occurrence of the perithecial stage in nature is confirmed, our present concept regarding the survival of the Red Rot fungus under Indian conditions may have to be modified. According to Chona (1950) and Chona and Nariani (1952), the conidial stage of the fungus can survive



Fig. 1—A perithecium of $P.\ tucumanensis$ (longitudinal section) from autoclaved leaf-sheath (x 360)



Fig. 2.—Ascigerous mass from a perithecium of P. tucumanensis (x360)

in unsterilised soil or soil-compost mixture for a period not exceeding three months. The capacity of the perithecial stage to survive under field conditions needs further investigation.

The authors wish to record their grateful thanks to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology, for his keen interest, encouragement and the facilities provided for this work.

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THE SIXTH ANNUAL REPORT OF THE INDIAN PHYTOPATHOLOGICAL SOCIETY (1952).

I am presenting herewith the Sixth Annual Report for the year 1952 of the Indian Phytopathological Society. The Society suffered a great loss in the death of Dr. B.B. Mundkur who was chiefly responsible in bringing the Society into existence. It was due to his labours that the Society has reached its present status.

During 1952, six members were enrolled, of whom one is a lifemember. By the end of 1952, the total membership stood at 165, of whom one is a Patron, 37 are life-members who have paid their dues in full and two life-members are paying their subscriptions by instalments. Seventy-one members have paid their 1952 dues and 54 members have yet to pay their 1952 subscriptions. I hope these members will pay their subscriptions at an early date.

During this year, Volume III, No. 2 and Volume IV Nos. 1 & 2 of the INDIAN PHYTOPATHOLOGY were published. The Journal continues to enjoy great popularity in foreign countries and 15 new subscribers have been added to the list bringing the total to 122.

Putting aside a sum of Rs. 5,000/- invested in National Savings Certificates, the year began with Rs. 6,575/7/7 to our credit. Receipts during the year amounted to Rs. 4,280/3/11, which include Rs. 250/- given by the National Institute of Sciences of India as donation and Rs. 1,058/3/- by the Indian Council of Agricultural Research as subsidy towards the printing charges of the Journal. The expenses incurred during the year have amounted to Rs. 5,825/12/3 only. During this year, payment had to be made to the Press for three issues instead of two. On the whole, our financial position is quite sound.

The accounts for 1951 were audited by a Chartered Accountant at a cost of Rs. 25/- and are placed before you. They will, as before, be published in the next issue of the Journal. The accounts for 1952 will be audited and it is hoped that a statement of receipts and expenditure will be sent to the members by the end of January, 1953.

Our grateful thanks are due to the Indian Council of Agricultural Research for subsidizing the printing of the Journal and to the National Institute of Sciences of India for the grant of Rs. 250/-.

I take this opportunity to express my grateful thanks to the members of the Society, the counsellors and late Dr. B.B. Mundkur, who was our President, for the support, encouragement and advice which I have received from them. My grateful thanks are also due to Dr. R.S. Vasudeva for giving me the necessary facilities for my work.

THE INDIAN PHYTOPATHOLOGICAL SOCIETY, DELHI.

Receipts and payments account for the year ended 31st December 1952.

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We We have examined the annexed Receipts and Payments Account for the year ended 31st December 1952. have to report as follows:--

- In respect of the twelve years Post Office National Savings Certificates, we have been furnished with a certificate from Lloyds Bank Ltd., New Delhi, in support of the Safe Custody with them of these Certificates on 31 December 1952.
- In 1951, a sum of Rs. 100/- was advanced to Dr. Mundkur towards incurring expenses on behalf of the us a letter written by Dr. Mundkur on 22.4.1952 together with a set of vouchers supporting expenditure of Rs. 70/9/6. In the statements accompanying this report the sum of Rs. 70/9/6 has been accounted for, The Council will have to take steps for the recovery of the balance of Rs. 29/6/6 or write off the amount Society. We are informed that Dr. Mundkur died in December 1952. Your Hony, Secretary has furnished as irrecoverable.

Subject to these remarks we certify that the annexed Receipt and Payments account of your Society for the year ended 31st December 1952 has been found correct in accordance with the books and information supplied to us.

New Delhi, Dated: 2nd July, 1953

R. PRASADA Secretary-Treasurer

AIYAR & Co. Chartered Accountants



INDIAN PHYTOPATHOLOGICAL SOCIETY

Instructions to Authors

Membership in the INDIAN PHYTOPATHOLOGICAL SOCIETY is pre-requisite to publishing in INDIAN PHYTOPATHOLOGY but the Editorial Board may relax this rule in the case of contributions of exceptional merit and communicated with a special recommendation by a member. The Editorial Board may invite distinguished scientists to

contribute articles of interest to the Society.

Contributions should be on one side of the page, double spaced, with a 1-1/4th inch margin on the left. In form and style, such as punctuation, spelling and use of italies, the manuscript should conform to the best Journals in the U.K. and U.S.A. Authors should strive for a clear and concise style of writing. The name and address of the Institution at which the work was done should be cited immediately after the SUMMARY at the end of the article on left hand side. Tables should be numbered and each table should have a heading stating briefly its contents. References to literature should be made as foot notes only when four or fewer citations are given. If there are more, they should be listed under 'REFERENCES' at the end of the paper and referred to by date in brackets in the body of the article. Citation should give the name of the author (or others), his (or their) initials year of publication and then the full title correctly, followed by the name of the Journals, number of the volume, a colon and page numbers. If the title is in a foreign language, then diacretic signs and capitalization should be precisely as in the original. The names of the Journal should be as abbreviated in the WORLD LIST OF PERIODICALS, 2nd Ed., 1934, but as that book may not be available to all, contributors are requested to give the titles in full. Abbreviating will in that case, be done by the Editors. If an article has not been seen in original, then that fact should be clearly stated. An example citing is given below:-

Conovor. R.A. (1948).....Studies of two viruses causing mosaic diseases of soybean *Phytopathology*, **38**: 724-735.

Because of high cost of half-tone blocks carefully made line drawing on Bristol board in black ink will be preferred. Photographs when necessary should be printed on glossy contrast paper and be of best quality. Full page figures and photographs should be made to reduce 4×6 1/2 inches, the standard size for all plates. Each author is allowed one page of half-tone illustration for each article or its equivalent, and the cost of half-tone blocks and paper in excess will be charged to author. Drawings must be drawn to standard scales, so that they can be compared with one another. e.g. $\times 10$, $\times 50$, $\times 100$, $\times 250$, $\times 500$ etc. It is not always possible to get a magnification at a round figure with a camera lucida but the printer can readily reduce drawings at any magnification to the standard, provided a scale is added to the drawing. The scale should measure from 5 to 10 cm. the longer the better and the printer should be instructed to reduce this line to the desired magnification.

Authors are invited to consult Bisby's 'An Introduction to Taxonomy and Nomenclature of Fungi' (1945), pp. 38-41 and Riker's 'The preparation of manuscripts for *Phytopathology*, *Phytopathology* **36**: 953-977, 1946, before

preparing their mss. and figures.

Articles will be published in the order of their approval for publication but the address of the retiring President and invitation articles will be published when received.

To comply with the International Rules of Botanical Nomenclature, Latin descriptions must be supplied to validate new species and genera.

Authors requiring reprints with or without covers should place an order for the copies wanted at the time of returning the proofs and they will be charged actual cost.

INDIAN PHYTOPATHOLOGICAL SOCIETY

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